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PROCEEDINGS OF THE BIOCHEMICAL SOCIETY

The **247th Meeting** *of the Biochemical Society was held at the British Postgraduate Medical School, Ducane Road, Hammersmith, W.* 12, *on Saturday,* 19 *January* 1946, *at* 11.30 *a.m., when the following papers were read:*

COMMUNICATIONS

Observations of the Biochemical Behaviour of Paludrine and some New Antimalarials. By J. MADINAVEITIA

The Pharmacology of Paludrine and some other New Antimalarials. By A. SPINKS, MARY M. TOTTEY and B. G. MAEGRAITH

The absorption, distribution and excretion of 3349 (2-*p*-chloroanilino-4-β-diethylamino-ethylamino-6-methylpyrimidine dihydrochloride), 4430 (N_1-*p*-chlorophenyl-N_5-methyl-N_5-isopropylbiguanide acetate) and paludrine (N_1-*p*-chlorophenyl-N_5-isopropyl-biguanide hydrochloride) have been examined in the rat. In this species, and probably also in the rabbit, they show pharmacological properties qualitatively similar to those of mepacrine: (1) Concentrations in blood and plasma are low. (2) Concentrations in blood are two to four times those in plasma. (3) Concentrations in glandular tissue and lung are many times those in plasma. (4) Excretion in the urine continues for several days, and the total amount excreted therein is less than 25 % of that administered. (5) Considerable amounts are excreted from the blood into the intestine and removed from the body in the faeces. In each of these respects 3349 shows the closest resemblance to mepacrine. Maximum concentrations given by 100 mg. of the drugs/kg. orally in the rat are: mepacrine 0·6, 0·3; 3349 0·8, 0·25; 4430 1·5, 0·5; paludrine 2·0, 0·8 mg./l., in blood and plasma respectively. Glandular tissue/plasma concentration ratios in the rat are: mepacrine 300–1000; 3349 100–300; 4430 and paludrine 10–50. The ratio of faecal to urinary excretion following intravenous administration is much greater for 3349 than for 4430 and paludrine.

Thus, the dialkylaminoalkylamino side-chain of 3349 confers very similar properties to that of mepacrine, and these properties probably reflect the ability of the two drugs to reach the malaria parasite in high concentration. Although 4430 and paludrine differ quantitatively from 3349 and mepacrine, the strong qualitative similarity suggests that the novel side-chains of these drugs have a dual function, first conferring activity by virtue of their hetero-enoid structure (Curd & Rose, 1946), and secondly, conferring favourable pharmacological properties, possibly by virtue of their highly basic character.

The absorption and excretion of paludrine have also been examined in human patients and volunteers. Following single and therapeutic doses of 50 and 500 mg., up to 50 % was excreted in the urine. Plasma concentrations of about 500 μg./l. were reached on therapeutic doses of 500 mg. twice daily after 4–5 days. On 50 mg. twice daily the plasma concentration was about 100 μg./l. After 14 days on 500 mg. twice daily paludrine could be detected in the urine for 9 and in the plasma for 6 days.

REFERENCE

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A Rapid Method for the Estimation of Paludrine in Urine. By J. C. Gage and F. L. Rose

A method for the estimation of paludrine in urine has been developed, based on the ability of the drug to form an organo-metallic complex with copper. Urine is treated with ammoniacal copper sulphate and the complex is extracted with benzene; the copper in the benzene layer is estimated colorimetrically with sodium diethyldithiocarbamate. The method is simple and rapid and capable of estimating, within the limits of accuracy required, the concentrations in urine arising from clinical administration of paludrine.

Estimation of Paludrine. By E. J. King, I. D. P. Wootton and M. Gilchrist

Treatment of Caecal Coccidiosis of Chickens by Sulphonamides. By F. D. Asplin, E. Boyland and C. Horton-Smith

Caecal coccidiosis in chickens produced experimentally by infection with *Eimeria tenella* can be effectively controlled by treatment with certain sulphonamides. Sulphanilamide, sulphapyridine and sulphathiazole, however, have no curative action on the disease. Sulphathiazole is not easily absorbed so that blood concentrations which might affect the parasite are not attained by oral dosing. Sulphapyridine, on the other hand, is absorbed into the blood and must therefore be assumed to have no direct action on the parasite. Sulphapyrazine, sulphamezathine, sulphamerazine and sulphadiazine all have curative action when added as sodium salts to the drinking water of infected chicks and the activity varies with the blood concentrations which are obtained. In winter the blood concentration shows considerable diurnal variation owing to a fall in intake during the long periods of darkness. Optimum therapeutic results are obtained when the minimum blood concentration is over 5 mg./100 ml. This is regularly obtained when chicks have access to 0·1 % sulphapyrazine or 0·2 % sulphamezathine in place of drinking water. Sulphadiazine is not so well or so regularly absorbed so that concentrations of 5 mg./100 ml. cannot be regularly attained and the therapeutic results are less satisfactory with this compound. High blood levels can be obtained with sulphaguanidine when this is administered as a 1 or 2 % constituent of the chicks' food. In spite of the blood concentrations obtained sulphaguanidine has only a prophylactic action and will not cure existing disease.

The differences in absorption of the various drugs are difficult to explain. Sulphapyrazine for instance gives higher blood concentrations in 2-week-old chicks than in 12-week-old chicks. Sulphapyrazine and sulphaguanidine appear to be more readily absorbed by chickens than by mammals.

The curative effect of sulphamezathine and sulphapyrazine is completely neutralized when *p*-amino benzoic acid (1/20 of the molar concentration of the sulphonamide) is added to the drug solution.

In practice, outbreaks of caecal coccidiosis in poultry are best treated by addition of 0·1 % sodium sulphapyrazine or 0·2 % sulphamezathine to the drinking water. Treatment should not be continued for more than 7 days or symptoms of vitamin deficiency, presumably due to sterilization of the gut, may occur.

If young chicks are dosed with sulphamezathine over a period of two or more weeks the blood clotting time is lengthened, presumably on account of decreased synthesis of vitamin K in the gut. In a few cases multiple petechial haemorrhages of the intestines may be found on post-mortem examination after prolonged dosing. Dosing with

sulphapyrimidines, particularly sulphamezathine, causes hyperplasia of the seminiferous tubules of the testis in cockerels. The testicular enlargement is accompanied by precocious development of the comb and wattles. The effect on the testis is not produced by sulphapyrazine, sulphathiazole or thiouracil. Sulphamezathine does not appear to have any such comparable stimulating action on the testis of rats.

Excretion Products of Mepacrine: a Spectrophotometric Study. By E. J. King, M. Gilchrist and A. L. Tarnoky

Large quantities of human urine from volunteers on mepacrine therapy were extracted at alkaline pH with ethylene dichloride. After an extraction with strong soda, the basic material was taken through acid and benzene phases and chromatographed on alumina. The soda extract was similarly treated. Elution gave seven fractions. After purification by repetition of the distribution and chromatography, spectrophotometric curves of the fractions were obtained in the range 2300–5000 A. at different pH's. Mepacrine, acridine, and several acridine and diphenylamine derivatives were spectrographed for comparison.

The spectrophotometric evidence indicated that two of the fractions (the greater part of the material) consisted of unchanged mepacrine. This was confirmed by mixed melting-points of their picrates with mepacrine monopicrate. The remaining fractions gave curves similar to derivatives of diphenylamine, and it was concluded that in these fractions a splitting of the acridine nucleus had probably occurred.

Several synthetic compounds in which the ring structure is intact and which might represent possible degradation products of mepacrine in the body failed to give spectrographic curves similar to any of our fractions or to any of those fractions of Scudi & Jelinek (1944) (with the exception of unchanged mepacrine) for which spectrographic curves are shown; the curves for 2-chloro-5-amino-7-methoxy (and -hydroxy-)-acridine are not similar to those of Scudi & Jelinek's fractions B_2 and C. Thus fractions 'B_2' and 'C' isolated by Hammick & Mason (1945) from human urine, which they consider identical with the above two acridine compounds respectively, may not be identical with the corresponding Scudi & Jelinek fractions.

In the course of this work nine acridine derivatives (including acridones and 5-amino-acridines with nuclear substituents such as methoxy-, hydroxy- and chloro-) were examined spectrophotometrically. The curves generally have an intense absorption band in the region 2600–2800 A. and a wide band of lower intensity at about 4200 A. Some of the compounds also show low absorption maxima at an intermediate wave-length. The presence of this latter band is believed to be connected with the existence of a quinonoid structure due to the imino form of 5-amino-acridines or the keto forms of acridones or hydroxy compounds. Hydroxy compounds also show a characteristic shift to longer wave-lengths of the 4000 A. band as the pH is increased. The curves of all acridine compounds studied showed isobestic points.

No isobestic points were found in the two diphenylamine carboxylic acids spectrographed. Their absorption curves can best be described as distorted acridine-type spectra, with a strong band in the low wave-length region and the other bands much displaced towards lower wave-lengths. These compounds were found to deviate considerably from Beer's Law.

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Further Studies on the Antibacterial Action of Substituted Aminophenols. By M. BARBER and G. A. D. HASLEWOOD

In a previous study (Barber & Haslewood, 1945) on the antibacterial activity of a series $R.C_6H_4.NH_2(3).OH(4)$, the compounds tested were primary amines. We have now made and tested the following secondary amines:

2- and 4-methyl, 2- and 4-ethyl, 2- and 4-*n*-propyl,
2- and 4-*iso*propyl, 4-*n*-butyl, and 4-*sec.*-butyl aminophenol.

All these compounds differ in action from the primary amines previously tested and from most other antibacterial agents in that all are at least as effective *in vitro* against *Ps. pyocyanea* as against *Bact. typhosum* and some have a stronger action against the former organism.

The compounds of the series

were the most active, all of them having an activity *in vitro* of the same order as 2-aminophenol against *Bact. typhosum* and *Staph. aureus* and being considerably more effective than this compound against *Ps. pyocyanea*. Adrenaline and *p*-hydroxyphenol glycine ($R=CH_2COOH$) were found to have a similar action.

The antibacterial activity of all these compounds is increased by oxidizing agents such as hydrogen peroxide and reduced by ascorbic acid. They are also methaemoglobin formers and *in vitro* blood reduces their activity.

REFERENCE

Barber, M. & Haslewood, G. A. D. (1945). *Biochem. J.* **39**, 285.

Isolation of Purified Fractions of the Anti-Pernicious Anaemia Factor from Liver. By W. B. EMERY and L. F. J. PARKER

Two fractions have recently been isolated from liver by a modification of the Laland & Klem (1936) process, using boiling 65% ethyl alcohol for elution of a charcoal adsorbate. After concentration *in vacuo* to remove alcohol, the solution was readsorbed on a smaller amount of charcoal, the bed washed and then further eluted with boiling 65% alcohol. This solution, after concentration *in vacuo*, has given a fraction active as defined by Emery & Hurran (1945) in human pernicious anaemia on a single dose of 3 mg. Further fractionation was carried out by means of ammonium sulphate precipitation, solution in glacial acetic acid, precipitation with ether, solution in phenol and extraction with water after dilution of the phenol extract with ether. The final fraction was obtained in a yield of 1·2 mg. from each kilogram of liver and has proved active as defined by Emery & Hurran (1945) in human pernicious anaemia on a single dose of 1 mg.

This product has been dried from the frozen state and shown to have no specific ultra-violet absorption characteristics and to be laevo-rotatory in aqueous solution. Chemical examination of the fraction is now under way.

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Inhibition of Urease by Cu in the Presence of Ascorbic Acid and Related Substances. By L. W. Mapson (introduced by L. J. Harris)

Elson (1943) found that urease is inhibited by small amounts of ascorbic acid and that this inhibition is eliminated in the presence of cysteine. Quastel (1943) suggested that the inhibition was due to a reaction between dehydroascorbic acid and an —SH group of the enzyme, and that the protective effect of thiol compounds was due to the fact that they reduced dehydroascorbic acid. Giri & Seshagiri Rao (1944), however, did not find that dehydroascorbic acid inhibited urease, and since they observed that there was no inhibition of the enzyme by ascorbic acid in the presence of substances which form complexes with Cu (8-hydroxy-quinoline and diethyl-dithio-carbamate), they suggested that the inhibition might be due to the formation of intermediary substances like Cu_2O, produced during the oxidation of the vitamin.

Our experiments show that Cu^+ is a much more effective agent than Cu^{++} in inhibiting urease, and that ascorbic acid acts as an inhibitor only in so far as it reduces $Cu^{++} \rightarrow Cu^+$. The experimental findings on which this evidence is based are as follows:

(*a*) Ascorbic acid does not inhibit urease when Cu is absent, but does so when Cu is present.

(*b*) The inhibition by Cu^{++} plus ascorbic acid is of the same magnitude as with Cu^+ alone. The inhibition produced by Cu^+ is not increased by the addition of ascorbic acid.

(*c*) Other reducing substances containing a dienol group and capable of reducing $Cu^{++} \rightarrow Cu^+$, e.g. hydroxytetronic, reductic and dihydroxymaleic acids, resemble ascorbic acid in their power to inactivate urease in the presence of Cu.

(*d*) Urease inactivated either by Cu^{++} plus ascorbic acid or by Cu^+ alone may be reactivated by H_2S, or by cysteine.

Dehydroascorbic acid does not inhibit urease, even in the presence of Cu. 2, 3-Diketogulonic acid (produced by mutarotation when solutions of dehydroascorbic acid are left standing) inhibits only in the presence of Cu, but to a less extent than does ascorbic acid.

Of the metallic salts tested, Cu^{++} and Au^{+++} alone had their toxicity increased by the addition of ascorbic acid. With Hg^{++} salts the toxicity was reduced by ascorbic acid.

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Relation between Glycolysis and Tissue Integrity in Gastric Mucosa. By C. Lutwak-Mann and A. M. Barrett

The glycolysis of the gastric mucosa (rat) was studied manometrically and chemically using complete transverse sections (10–15 mg. dry weight) of the stomach usually in the region of the cardiac glands. This technique was adopted because if the mucosa was separated from the rest of the wall it rapidly disintegrated, with loss of protein into surrounding saline and a fall in enzymic activity; stomach wall devoid of mucosa was almost without glycolytic and oxidative activity.

Anaerobically, the gastric mucosa readily produced lactic acid provided that glucose was added. The rate of glycolysis remained steady for 3–4 hr. (0·6 mg. lactic acid per 10 mg. dry weight per hr.). The Ringer-bicarbonate solution remained clear after the completion of a 4 hr. experiment and contained hardly any soluble protein. However, when the gastric sections were kept anaerobically without added glucose, then no lactic acid was formed while the mucosa suffered visible damage, the saline became opalescent and contained a great deal of soluble protein.

The changes brought about by aglucotic anaerobiosis were irreversible. When two gastric sections were incubated anaerobically for 2 hr. at 38°, one with, one without glucose, and both were next transferred to fresh Ringer-bicarbonate containing glucose, then in the first instance glycolysis of the mucosa was unimpaired, while in the second the ability to glycolyze was lost and there was progressive disintegration of tissue.

Histological studies showed that in the presence of glucose the epithelium lining the stomach, and the gastric pits usually remained intact, whereas without glucose many or all of its cells were shed. Changes in the gastric glands occurred both in the presence and absence of glucose, but were rather more severe in its absence.

M/400-glucose sufficed to preserve the gastric mucosa anaerobically. Only mannose could be substituted for glucose, while fructose, galactose, glycogen, lactate, pyruvate, hexosephosphates, also cozymase, ATP, nicotinamide, guanosine, all were unable to check the damage to the mucosa and prevent the loss of glycolytic activity due to asphyxiation in absence of glucose.

Aerobically, the gastric mucosa was independent of glucose; it showed substantial O_2 uptake without addition of substrate. The respiration of the mucosa was little affected by a preceding period of glucose-free anaerobiosis.

DEMONSTRATIONS

Estimation of Paludrine. By E. J. King, I. D. P. Wootton and M. Gilchrist

The Estimation of Small Amounts of Glycogen in Tissue. By V. M. Walshe

Iodometric Estimation of Plasma Bicarbonate. By E. J. King, I. D. P. Wootton and R. L. F. Tupper

A Comparison of Laevorotatory and Dextrorotatory Quartz on the Lungs of Rats. By E. J. King, N. Rogers, M. Gilchrist and G. Nagelschmidt.

The **248th Meeting** *of the Biochemical Society was held in the London School of Hygiene, Keppel Street, London, W.C.* 1, *on Saturday,* 16 *February* 1946, *at* 11 *a.m., when a discussion took place on 'Amino Sugars and Uronic Acids in Nature', and the following communications were given:*

Some Aspects of the Physiology and Pathology of the Natural Hexosamine and Uronic Acid Compounds. By Gunnar Blix (introduced by W. T. J. Morgan)

The physiological importance of hexosamines and uronic acids is evident already from their wide distribution in living nature.

As components of long-chain *polysaccharide acids* they appear in vertebrates, especially in the extracellular ground substance of connective tissues and in secretions of mucous membranes and glands.—Chondroitin sulphate and hyaluronate seem to function as *cementing material* in the connective tissues, probably in the form of an ultra-structural network of gel nature. Softness and decrease of pressure elasticity of cartilage have been

found to be attended by a reduced content of chondroitin sulphate (Hirsch, 1944).—The 'spreading effect' of hyaluronidase clearly indicates an important cementing function of hyaluronate in the subcutaneous tissue. There is some experimental evidence of the hyaluronate having a similar function in the capillary walls.

The polysaccharide acids show under special conditions a marked ability to form gels in combination with proteins. It is, however, doubtful if the vitreous body represent such a gel, since it is not destroyed by hyaluronidase.—The material cementing the follicular cells around the newly ovulated egg, functioning at the same time as an enveloping *protective gel*, seems to contain hyaluronate as an essential constituent. This gel is disintegrated by the hyaluronidase of the spermatozoa, a process of considerable physiological interest. From a comparative physiological point of view it is noteworthy that the hyaluronic acid may form a protecting gel envelope also around certain bacteria.

The high viscosity of polysaccharide acid solutions makes them suitable as biological *lubricants*. The synovial fluids contain only about 0·02–0·05 % hyaluronate, but they often owe 80–90 % or more of their marked viscosity to this substance. The occurrence of hyaluronidases or hyaluronate in the bacteria most often found in infectious rheumatic disease and the frequent occurence of the pathological changes in this disease in tissues containing hyaluronate have raised the still unanswered question whether these circumstances are causally connected.

The secretions of the mucous membranes and glands owe their characteristic physical properties mainly to polysaccharide acids, of which the mucoitin sulphuric acid is the best known. These secretions form on the mucous membrane surface a slimy layer with lubricating as well as generally protective functions. The protective properties of mucin should be considered also in the case of the teeth. The marked inhibitory effect of the polysaccharide acids on the action of pepsin and trypsin is likely to form an important part of the mechanism which prevents auto-digestion of the mucosa. The physiology and pathology of the mucous secretions deserve a greater attention than that hitherto bestowed upon them.

A substance which is metachromatically stained by toluidine-blue has been demonstrated to be present in relative abundance in all growing mesenchymal tissues, especially in embryonic connective tissue, granulation tissue and malignant mesenchymal tumour tissue (Holmgren, 1940; Sylvén, 1941, 1945). The staining method used is believed to be specific for polysaccharide sulphuric acid esters.

Neutral polysaccharides composed of *N*-acetylated glucosamine and hexoses often occur firmly bound to protein. These carbohydrates seem to have a much lower molecular weight than the polysaccharide acids and to differ widely from them in physical properties and physiological functions.—In blood serum the α- and β-globulins are particularly rich in such carbohydrates. The α-globulin is increased in acute infections and in certain other diseases, and this increase is paralleled by a rise of the glucosamine content of serum. The significance of these changes is as yet unknown.

Some glycoproteins containing neutral polysaccharide are found to be endowed with highly specialized functions. To this group belong the gonadotropic pregnancy hormone and probably also the choline esterase recently isolated in crystalline form from blood serum. The role of the carbohydrate group for the physiological action of these substances is not known.—A number of serologically highly important compounds, the physiological significance of which still remains obscure, are also glycoproteins of this type, namely, the blood-group antigens, the Forssman hapten and a fraction of the complement.

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Polyuronides. By E. G. V. PERCIVAL (introduced by J. N. DAVIDSON)

The term polyuronide, taken literally, would describe a polysaccharide made up entirely of uronic acid residues. This definition is far too narrow and any homogeneous polysaccharide containing uronic acid units among its building stones may be classed as a polyuronide. In this brief review attention will be paid to the chemical constitution of a few representative polyuronides, although it must be admitted that in many cases our information is far from complete.

First we have the 'full' polyuronides, pectic acid and alginic acid. Although pectins vary widely in properties according to their origin, it appears that the variations are connected with the associated arabans and galactans rather than with the essential backbone, pectic acid, which is composed of *d*-galactopyruronic acid units mutually united by 1:4-α-linkages (K. P. Link, E. L. Hirst, F. Smith). Alginic acid, on the other hand, is represented as a chain of *d*-mannopyruronic acid residues joined by 1:4-β-linkages (G. Lunde, E. L. Hirst). The formulae of both polyuronides when written in the conventional manner resemble cellulose, but X-ray measurements show (W. T. Astbury) that the period along the fibre axis for alginic acid is 8·7 A. as against 10·3 A. for cellulose. This is explained by the bonds joining C_1 and C_4 to the glycosidic oxygens taking up the alternative position in alginic acid to give a more irregular structure than is the case for the almost flat cellulose model. It is also believed that pectic acid is similar to alginic acid in this respect.

A polyuronide, representing a highly important group, is the specific polysaccharide of *Pneumococcus* Type III, which is constructed of cellobiuronic acid residues joined by β-linkages through the 1:3-positions (W. F. Goebel). The capsular polyuronide of *Pneumococcus* Type VIII also yields cellobiuronic acid on hydrolysis, but in this case the proportion of glucose to glucuronic acid is approximately 3 : 1 instead of 1 : 1. Less is known about the constitution of other type-specific polyuronides, e.g. of *Pneumococcus* Types I and II, *V. cholerae*, etc., but the capsular polysaccharide synthesized by *Rhizobium radicicolum* bears a resemblance to the type-specific polyuronides of *Pneumococcus* in its precipitin reactions. Furthermore, cellobiuronic acid is concerned in its construction (M. Stacey). Mention may be made also of the oxycelluloses prepared by the oxidation of cellulose with nitrogen dioxide. These substances may be termed in a sense 'artificial polyuronides', and it has been shown that they bear some relation to the specific polysaccharides of *Pneumococcus* (Types III and VIII) in their precipitin reactions (M. Heidelberger).

Precise knowledge of the constitution of the somewhat ill-defined polyuronide hemicelluloses is almost non-existent, owing to the difficulty of isolating homogeneous products. In many cases the uronic acid constituent has not been identified with certainty, e.g. the methoxyuronic acid of the hemicellulose of cottonwood, even though the proportion of uronic acid is relatively high in this instance, the polysaccharide consisting of a chain of 7–9 xylose residues (E. Anderson).

As much interest is attached to the mode of union of the other sugar units present in the polyuronides as to the way in which the uronic acid is joined, especially when the proportion of uronic acid is small. An investigation of the methylated glucoses obtained by the hydrolysis of a methylated hemicellulose fraction of Iceland moss revealed the existence of glucose residues linked through the 1:3, 1:4, 1:6 and the hitherto unreported 1:2 positions. Mannose, galactose and glucuronic acid were also detected in the products of hydrolysis of the hemicellulose (or mixture of hemicelluloses). These results emphasize the complexity of the problems of hemicellulose chemistry.

Many mucilaginous polysaccharides contain uronic acid units, and such compounds have highly complex structures. Mention may be made of the seed mucilages of the plantains. The mucilage from *Plantago lanceolata* is found to have a high proportion of xylopyranose end-groups (isolated as trimethyl xylopyranose), a small proportion of galactopyranose end-groups, xylopyranose units linked by the unusual 1:2-linkage

(isolated as 3:4-dimethyl xylopyranose), galactopyranose units linked as in agar (1:3), galacturonic acid, and an as yet unidentified methylpentose. *Plantago arenaria* seed mucilage differs from the above in detail, but also possesses a highly branched structure. This mucilaginous polyuronide contains a high proportion of xylopyranose and a small proportion of galactopyranose end-groups. Xylopyranose units linked through the 1:2-positions, galacturonic acid, and arabinose residues are also present. The high proportion of 2-methyl xylose isolated from the hydrolysis products of the fully methylated polysaccharide makes it evident that some *d*-xylose units are triply bound to other residues.

The most precise information about complex polyuronides has been afforded by the masterly studies on gum arabic by F. Smith, and of other plant gums by E. L. Hirst. These substances have a relatively resistant core in which glucuronic acid is incorporated together with other sugar residues, e.g. in gum-arabic galactopyranose, in damson gum galactopyranose and mannopyranose. By a study of the aldobionic acids obtained from these 'degraded' gums, an idea of the structure of the skeleton has been arrived at. Attached to this core are other sugar residues easily removed by auto-hydrolysis, e.g. in gum-arabic *l*-rhamnopyranose, *l*-arabofuranose and 3-galactopyranosido-*l*-arabofuranose; in damson gum *l*-arabofuranose units are found as end-groups. Experiments of this kind have enabled formulations to be suggested for polyuronides of this type.

Gum tragacanth is even more complex, and the tragacanthic acid portion contains *d*-galacturonic acid units which are united as in pectic acid. End-groups of *d*-xylopyranose and *l*-fucopyranose are present as well as xylopyranose units linked through the 1:2-positions (Smith). Gum tragacanth also contains a neutral polysaccharide believed to be a complex araban associated with the tragacanthic acid, as well as a fraction which is steroid in nature.

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The Mode of Linkage of the Amino Sugars in their Naturally Occurring Derivatives. By M. STACEY

Amino sugars, chiefly *d*-glucosamine, occur widely in polymeric form as protein-carbohydrate complexes in body tissues and fluids, in the protective covering of Crustacea and in the protoplasm of micro-organisms, etc. In the carbohydrate residue the amino sugar may form the sole constituent unit as in chitin; it may occur with hexoses only, as in the blood group substances, etc.; or it may occur in combination with hexuronic acid as in heparin and hyaluronic acid. It would appear, with the possible exception of heparin, that the amino groups are not engaged in polymeric linkage but are always substituted by acetyl or other residues; also in the uronic acid-containing compounds the carboxyl groups are mainly free.

The protein-carbohydrate complexes have been described in the literature as mucins, mucoids, glycoproteins, glycoids, etc., and early work has been reviewed by Levene (1926). Karl Meyer (1938) has reclassified the compounds and his classification has been extended by Stacey (1943). In approaching structural problems in the group it must be emphasized that the nature of any complex is sharply affected by its mode of isolation, and such attempts as have been made to determine carbohydrate structure have been mainly carried out on relatively stable and often degraded fragments.

Although there are theoretically possible far more hexosamines than hexoses, so far only two naturally occurring ones have been isolated and characterized. These are *d*-glucosamine (chitosamine or 2-amino-*d*-glucopyranose) and chondrosamine (2-amino-*d*-galactopyranose). These are usually separated in the form of their crystalline hydrochlorides from an acid hydrolysate of the polysaccharide. Only chondrosamine has been synthesized though a number of purely synthetic amino sugars or their derivatives have been made. The best known of these is 'methyl epiglucosamine', which is methyl-3-amino-β-*d*-altrose. Others are 1-amino glucose (glucosylamine) and 1-amino sorbitol (glucamine) and derivatives of 2-amino altrose, 3-amino glucose, 3-amino idose, 4-amino mannose, 6-amino glucose, etc.

The close relationship between the amino sugars and the anhydro sugars has enabled the precise configuration of glucosamine and chondrosamine to be settled (Haworth, Lake & Peat, 1939) and the synthesis of chondrosamine to be accomplished (James, Smith, Stacey & Wiggins, 1945). Quite recently the reversible conversion of amino sugars into anhydro sugars has been carried out (Wiggins, 1946). It should be noted that the structure of glucosamine as a derivative of glucose was arrived at independently by purely X-ray crystallographic methods (Cox & Jeffrey, 1939).

Determination of the structure of polyamino-sugars by the methylation technique is not as generally applicable as with plant polysaccharides. With such chemically stable substances as chitin no ethers have yet been produced. Periodic titrations have also not so far proved successful (Wolfrom, Weisblat, Karabinos, McNealey & McClean, 1943; cf. Brown, Dunstan, Halsall, Hirst & Jones, 1945). With chitin, crystallographic studies have, however, been of particular value. From a study of chitin and a crystalline acetyl chitobiose derived therefrom, it was shown that the molecule consisted solely of *N*-acetyl glucosamine units linked through the 1:4-β-glycosidic positions. This showed the close relationship between cellulose and chitin which may, indeed, be regarded as 2-acetamido cellulose. Ovomucoid, a mucoprotein from egg white, contains a carbohydrate residue (20 %) which has been subjected to a fairly detailed examination (Stacey & Woolley, 1940, 1942). A method was devised whereby it was possible to effect deproteinization and methylation simultaneously to give an *N*-acetyl methyl compound in good yield. On hydrolysis this compound gave derivatives of *d*-mannose, *d*-galactose and *N*-acetyl *d*-glucosamine, the amount and nature of which indicated that by glycosidic attachment, seven *N*-acetyl glucosamine units and one galactose unit radiated from a central core of three mannose units. It was considered that this unique hendecasaccharide was not a repeating unit but was a whole molecule to the glucosamine units of which were attached peptide chains thereby constituting the mucoprotein. Support for the glycosidic mode of attachment of the glucosamine units to mannose was forthcoming from the work of Levene (1941), who isolated from a carbohydrate group of egg protein a glucosamine-mannose disaccharide. Reduction by hydrogen under pressure converted the mannose moiety into mannitol, thus revealing the formulation of the disaccharide as a glucosaminido-mannose. A serum mucoid has given a closely similar methylated carbohydrate residue (Stacey, 1946), and since there have been many reports in the literature of the isolation of glucosamine-mannose complexes from the prosthetic groups of serum and other proteins, including gonadotropic hormones, etc., it is likely that these carbohydrate residues are oligosaccharides also.

In a relatively stable carbohydrate residue of a blood group A substance from gastric mucin, which we have recently shown (Bray, Henry & Stacey, 1946) to be built up from *d*-mannose, *d*-galactose, *l*-fucose and *d*-glucosamine units, part of the latter are linked glycosidically as terminal residues. In an undegraded blood group A substance Morgan (1944) has shown that part of the amino-sugar constituents are bound in the complex by alkali-labile linkages.

There is as yet no precise knowledge regarding the mode of linkage of any of the units in hexuronic acid-amino sugar complexes such as heparin, mucoitin sulphate, etc. Ac-

cording to Wolfrom *et al.* heparin possesses an unusual structure, for they consider that the N atoms are neither free nor acetylated and speculate that they are engaged in polymeric linkages. In cartilage chondroitin sulphate, which appears to be the sole natural source of chondrosamine, the minimum repeating unit is a trisaccharide (Bray, Gregory & Stacey, 1944) composed of two molecules of glucuronic acid residues and one molecule of *N*-acetyl chondrosamine. One of the glucuronic acid residues is present as a terminal group, and it is clear that the chondrosamine residue is not linked glycosidically.

One interesting problem in the group concerns the *N*-acetyl residues both from the biological point of view in regard to their significance in immunity reactions, and from the chemical aspect in regard to their power of stabilizing glycosidic groups. Little is known in regard to the mode of synthesis of polyamino-sugars or their attached protein residues, and a wide field of research awaits the attention of chemists and biologists.

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Mucoitin Sulphuric Acid, Chondroitin Sulphuric Acid, Hyaluronic Acid and Heparin. By ANTOINETTE PIRIE

Acid mucopolysaccharides

Knowledge of acid mucopolysaccharides has been advanced in recent years by Meyer and his co-workers. Meyer (1938) suggested a reclassification of carbohydrate-containing proteins, dividing them into two main groups, mucopolysaccharides and glycoproteins. A mucopolysaccharide can be defined as a hexosamine-containing polysaccharide which occurs either free or loosely combined with protein. Hyaluronic acid, mucoitin sulphuric acid, chondroitin sulphuric acid and heparin form a subgroup of acid mucopolysaccharides.

Hyaluronic acid was isolated by Meyer & Palmer (1936) from vitreous humour and umbilical cord. It has since been isolated from other body fluids and tissues, from tumours and from group A haemolytic streptococci. It is the simplest of the acid mucopolysaccharides, being made up of equimolar parts of acetyl glucosamine and glucuronic acid. It can be extracted from tissues with water and forms extremely viscous solutions if it has been prepared without the use of alkali or precipitation by glacial acetic acid.

Mucoitin sulphuric acid is the monosulphuric acid ester of hyaluronic acid. It was isolated from gastric mucin by Levene & Lopez-Suarez (1916), who also thought that it was present in vitreous humour, Wharton's jelly, ovomucoid, serum mucoid, sclera and the cornea. Meyer & Chaffee (1940) isolated 1·8 % of mucoitin sulphuric acid from ox cornea, but were unable to isolate it from the other sources suggested by Levene. Mucoitin sulphuric acid is more difficult to extract from tissues and to separate from protein than hyaluronic acid and the final product is less viscous.

Chondroitin sulphuric acid was first isolated from cartilage by Mörner (1889). Meyer (1938) has since isolated it from umbilical cord tissue and spongy bone, and Meyer & Chaffee (1941) have prepared it from skin. Levene & La Forge (1914) characterized the hexosamine as galactosamine and showed that chondroitin sulphuric acid was made up of equimolar parts of acetyl galactosamine ester sulphate and glucuronic acid. Viscous preparations of chondroitin sulphuric acid are obtained if alkali is not used in extraction.

Heparin. Howell & Holt (1918), investigating the effect of tissue extracts in prolonging the clotting time of blood, extracted an active fraction from liver to which they gave the name of heparin. Jorpes (1935) first suggested that heparin is an acid mucopolysaccharide containing more than one ester sulphate group. It has been isolated from liver, lung, spleen, kidney and intestinal mucosa. It is not present in detectable amount in normal blood, but Jacques & Waters (1941) have isolated it from the blood of dogs in anaphylactic shock. Heparin reacts with basic dyes such as toluidin blue, giving a deep purple compound, and Jorpes, Holmgren & Wilander (1937) have traced its distribution in cells by this staining reaction. It is present in the mast cells of the body tissues, in aorta walls and cells lying alongside the capillaries.

The nature of the links that exist between acid mucopolysaccharides and proteins in the body is not known. The acid mucopolysaccharides are, in general, part of the structure of body tissues, and their isolation in solution may involve breakdown of their naturally occurring compounds. The state of the acid mucopolysaccharides in the tissues can be judged to some extent by the means which must be used to extract them and free them from proteins. The degree of degradation of the polysaccharide will also depend upon these methods. Hyaluronic acid may be extracted with water; chondroitin sulphuric acid and mucoitin sulphuric acid can only be extracted with disodium phosphate, strong solutions of salts or with alkali. Heparin is extracted with alkali after tissue autolysis. Alkali can depolymerize acid mucopolysaccharides and preparations made by alkaline extraction cannot be considered to be in their native state. Hyaluronic acid is also depolymerized by reducing substances in the presence of oxygen. Robertson, Ropes & Bauer (1941) found that ascorbic acid and copper together reduce the viscosity of hyaluronic acid. Hale (1944) showed that this action only took place in the presence of oxygen. This oxidative degradation of hyaluronic acid may also go on during extraction procedures. Blix & Snellmann (1944) report that hyaluronic acid prepared in the absence of oxygen shows anisotrophy of flow.

The acid mucopolysaccharides are co-precipitated with proteins in dilute acid; these protein compounds form the mucins and mucoids of synovial fluid, vitreous humour, etc. Basic proteins, such as globin and histone precipitate acid mucopolysaccharides at neutrality. Meyer (1938) considers that there is a salt linkage *in vivo* between the acid mucopolysaccharides and proteins, chondroitin sulphuric acid and mucoitin sulphuric acid forming stable protein salts which do not dissociate at neutrality and hyaluronic acid forming salts which are completely dissociated at neutrality.

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Glycoproteins. By C. RIMINGTON

By the term 'Glycoprotein' is generally understood a substance belonging to the protein class but containing carbohydrate groups in chemical combination as an integral part of the whole molecule. Such carbohydrate groups may be composed of hexoses, hexosamines and hexuronic acids, and with the latter are frequently associated sulphuric acid groups.

The classification proposed by Blix (1940) places the uronic acid-containing glycoproteins in the group of *acidoglycoproteins* of which chondroproteins form a subgroup, whilst under the heading *neutroglycoproteins* are collected those substances in which the polysaccharide complex is devoid of acidic units. In the present contribution I shall confine myself to the neutroglycoproteins and propose to examine the evidence that has been produced to support the contention that the carbohydrates they contain are integral constituents of the molecules.

Proteins prepared from animal or vegetable sources frequently give positive Molisch and similar colour reactions. The means of purification such as precipitation with salts, crystallization, etc., do not always serve to eliminate the carbohydrate material which may in some cases be present in large amount. What criteria can be applied to test the conclusion that such materials are glycoproteins in which the carbohydrate is bound by chemically definable linkages to the rest of the protein?

Certain criteria usually applied in the case of simple proteins are equally applicable to the problem, namely:

(1) Constancy of composition including constancy of carbohydrate content after successive recrystallizations.

(2) Electrophoretic homogeneity.

(3) Homogeneity in the ultracentrifuge.

(4) Independence of solubility upon the amount of solid phase.

(5) Constant potency, if the substance exerts a measurable physiological activity.

Some of the glycoproteins for which recognition has been claimed may now be discussed in the light of the above criteria.

Serum albumin. The presence of carbohydrate in serum albumin preparations was first demonstrated by Rimington (1929, 1931) who identified the sugar constituents as glucosamine, mannose and galactose. Serum albumin does not however obey criterion (4); in fact, Sorensen (1930) demonstrated that crystalline albumin could be separated into a series of crystalline preparations possessing differing solubilities, and according to the work of Sorensen & Haugaard in 1933, differing widely in carbohydrate contents. Sorensen concluded that apparently homogeneous crystalline serum albumin is in reality an association of mutually interacting component systems. These views have been strengthened by the subsequent work of others. Hewitt (1938) postulates the existence of three proteins in the albumin fraction, one of which 'crystalbumin' is carbohydrate free. Rimington & Van den Ende (1940) found that, according to the scheme of separation adopted, they could prepare albumins of regularly increasing or decreasing carbohydrate content. The most rigorous tests of homogeneity were applied by McMeekin (1939) to a carbohydrate-free serum albumin obtained by recrystallization of the sulphate. This was shown to satisfy criteria nos. 1, 2, 3 and 4. He also obtained (1940) a crystallizable albumin containing 5·5 % carbohydrate which was shown to be homogeneous in the electrophoresis apparatus and the ultracentrifuge but unfortunately criterion (4), that of independence of solubility on the amount of solid phase was not applied. In view of my own experience with gonadotrophin, to be described shortly, it would be very dangerous to assume that this albumin was a single pure substance.

Ovalbumin and ovamucoid. Following the earlier work of Levene & Mori (1929) on egg proteins, Neuberger (1938) investigated the carbohydrate moiety of crystalline ovalbumin and found that on repeated recrystallization of this protein the carbohydrate content

became constant and from such material a complex of 4 mol. of mannose with 2 mol. of glucosamine and a small quantity of an unidentified nitrogeneous constituent was isolated. The claims of ovalbumin to be considered a true glycoprotein would thus appear to be considerable, although the solubility test was not applied to the recrystallized material when constant carbohydrate content had been achieved.

Pituitary gonadotrophins. Somewhat surprisingly the protein hormones of the anterior lobe of the pituitary are among the most carefully purified and studied of all proteins. Two of these, the gonadotrophic hormones thylakentrin, or follicle-stimulating hormone, and metakentrin, or luteinizing hormone, are rich in carbohydrate, but the former has not yet been obtained as an electrophoretically homogeneous substance. Metakentrin, on the other hand, has been prepared from both sheep (Li, Simpson & Evans, 1942) and hog (Chow, Van Dyke, Greep, Rothen & Shedlovsky, 1942) pituitaries in a state satisfying all the criteria of purity listed above. The hormones were not however identical, that from the sheep having 4·5 % of carbohydrate as against 2 % in the hog hormone: differences also occurred in molecular weight and isoelectric points. The ratio of hexose to hexosamine was unity in each material.

Claims to have isolated a chemically pure gonadotrophin from pregnancy urine have also been made by Gurin and his collaborators (1940). The material, which contained galactose and hexosamine in the ratio 2:1 and 10 % total hexose, was electrophoretically and ultracentrifugally homogeneous, but no solubility study was made. Solubility tests are similarly lacking in the case of the gonadotrophin present in the serum of pregnant mares. The preparations of Li, Evans & Wonder (1940) were, however, stated to be electrophoretically homogeneous. Their hexose content was 14 %.

In a study of the purification of this hormone, Rimington & Rowlands (1944) readily produced material possessing an activity of 3500 i.u./mg. and behaving as essentially homogeneous in the Tiselius apparatus and ultracentrifuge. The hexose content was 11·5 %. From such observations it might have been suggested that the hormone was isolated in a state of purity. A further fractionation, however, by means of acid ethanol, removed large amounts of inert material and yielded products with a potency nearly four times as great, viz. 12,5000 i.u./mg. In these the hexose content was only very slightly raised to 15 %. Sufficient material has not been available to carry out solubility tests but it is quite clear that in such preparations from horse serum one must be dealing with a mixture or mutually reacting system of very closely related glycoproteins only some of which possess gonadotrophic activity. Had there been no such biological property wherewith to distinguish them the conclusion might well have been drawn from the similarity in chemical composition, including carbohydrate content of the subfractions, that the material migrating with a single boundary and sedimenting uniformly was one molecular species of glycoprotein.

To summarize: It would appear that in the case of hog and sheep pituitary metakentrins all available criteria of homogeneity have been satisfied and these must be admitted to contain neutral carbohydrate groups attached in some way within their molecules; the same may be true also of ovalbumin. In the case of the other proteins discussed, demonstration of homogeneity has been too incomplete or not sufficiently rigorous for them to be admitted as pure glycoproteins. Systems can occur in which mutually interacting glycoprotein components are present.

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Blood Group Substances. By W. T. J. MORGAN

Landsteiner and his pupils divided human bloods into four serological groups according to the presence or absence of the agglutinable substances A and B within the erythrocytes. The two main agglutinogens (A and B) occur either singly (group A or B) or together (group AB) in human red cells or are absent therefrom (group O). Until recently group O erythrocytes were considered as cells devoid of the A and B agglutinogens and were not identified by the possession of an agglutinogen of their own. According to Bernstein's theory of the mechanism of heredity of the specific blood group agglutinogens A, B and O, the existence of three allelic genes is postulated and implies that erythrocytes of the geneotype OO possess a specific O factor corresponding to the A and B agglutinogens. Direct evidence for the existence of a specific O-substance was, however, obtained only recently.

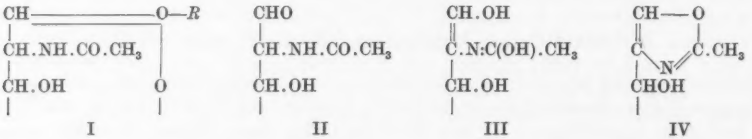
Many workers have attempted to isolate the blood group agglutinogens from human erythrocytes but the material isolated was in no instance obtained in sufficient quantity to establish its homogeneity or to investigate in any detail its chemical nature (see Wiener, 1943; Landsteiner, 1945). Perhaps little progress in determining the chemical nature of the blood group substance could now be reported but for the fact that these important biological materials are not limited to the erythrocytes but are present in the body fluids and secretions of the majority of persons irrespective of the A, B, O group.

An early observation (Schiff & Brahn, 1926) revealed that commercial peptone and pepsin contained a considerable quantity of group A substance, and subsequently a rich source of A substance was detected in hog gastric mucosa and identified as a neutral polysaccharide that contained *N*-acetylglucosamine and *d*-galactose (Meyer, Smyth & Palmer, 1937) and later shown to possess a component rich in amino acids (Landsteiner & Harte, 1940; Morgan & King, 1943). Besides *N*-acetylglucosamine and *d*-galactose the polysaccharide is said to contain *l*-fucose and *d*-mannose (Stacey, 1943).

A considerable amount of experimental evidence points to the labile nature of the specific blood group substances and it would appear that if their isolation is to be achieved with the minimum of change in the physical, chemical and biological properties carefully controlled methods must be employed. The use of such methods, some of which already have been employed for the isolation of bacterial antigens (Morgan, 1944), enabled undegraded preparations of the human A, B and O substances to be isolated from the fluids of pseudomucinous ovarian cysts (Morgan & van Heyningen, 1944; King & Morgan, 1944; Morgan & Waddell, 1945; and unpublished work).

The undegraded substances show a high viscosity at a concentration of 1 % in saline. Analytical figures vary within the limits C, 44·5–45·5; H, 6·5–6·7; N, 5·9–6·1 %. Acid hydrolysis gives rise to about 50 % reducing sugars, 33–37 % glucosamine, 4·5–5·0 % of α-amino N (Van Slyke) and 2·2–2·6 % α-amino acid N. These polysaccharide-amino acid complexes are degraded by heating at 100° (pH 7·0) with loss of viscosity and certain of their serological properties, and more extensive and rapid disruption of the substances is achieved by heating with dilute alkali (0·05 N-Na_2CO_3 for 3–4 min. at 100°). This mild

treatment with alkali causes a part of the *N*-acetylglucosamine molecules within the complex (I) to be changed so that they now possess a reducing aldehyde group (II) which, in the presence of alkali, presumably changes to the enolic structure (III) and eliminates water with the formation of a heterocyclic ring (IV). It is this 2:4 disubstituted oxazole (or oxazoline) structure (IV) which condenses with the *p*-dimethylaminobenzaldehyde and gives rise to the reddish purple coloration (Morgan & Elson, 1934; Morgan, 1936, 1938; White, 1940). The maximum colour developed is equivalent to 16–18 % of *N*-acetylglucosamine and represents rather less than half of the total glucosamine within



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the complex. Further heating with alkali causes considerable decomposition with destruction of the colour producing substance. Dialysis of blood group A substance after gentle treatment with alkali to yield the maximum *N*-acetylglucosamine colour with Ehrlich's reagent shows that the complex has been changed in such a manner that about 66 % of the total material diffuses through a cellophane membrane. The material which diffuses contains the major part of the components that give an immediate colour with Ehrlich's reagent (structure IV or the corresponding oxazoline) and which reduce Somogyi's copper reagent. The material retained by the cellophane membrane, on the other hand, contains at least 80 % of the total amino-acid N, and the remaining part of the glucosamine and carbohydrate. The complex which is electrophoretically homogeneous at *p*H 8·0 and 4·0 and sediments as a single component in the ultracentrifuge [sedimentation constant, $1{\cdot}6 \times 10^{-13}$ (C, 1·0 % Corr. to H_2O, 20°). Probable mol. wt. 17,000] is without reducing power until after acid hydrolysis. It is not possible to estimate the total glucosamine in the diffusate since the *N*-acetylglucosamine which has been converted to the oxazole derivative by the action of alkali cannot be hydrolyzed by acid to give glucosamine, for acid (0·1 N-HCl at 18°) treatment of the oxazole derived from *N*-acetylglucosamine gives rise to extensive decomposition without the formation of glucosamine. The alkali labile linkages would appear to be the glycoside linkages which are associated with C atom 1 of *N*-acetyl-glucosamine. *N*-acetyl and *N*-benzoyl methyl glucosaminides, however, are not hydrolyzed under the same conditions (0·05 N-Na_2CO_3 at 100° for 3–4 min.) and yield neither reducing sugars nor a colour with Ehrlich's reagent.

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Enzymic Degradation of Polysaccharides containing Amino Sugars and Uronic Acids. By H. J. ROGERS

The high weight and the configuration of polysaccharide molecules endows solutions of such substances as pectin, hyaluronate, etc., with a range of physico-chemical properties, changes in which can be observed during enzymic hydrolysis. Methods of estimation of the potency of enzyme preparations have perforce been based upon such changes selected in an arbitrary manner, e.g. the rate of fall in viscosity, or release of reducing sugars from the substrate. Owing to the paucity of knowledge of the exact structure of the undegraded polysaccharides, the chemical nature of the hydrolysis, which leads to the change measured, is often not known. Moreover, in some instances it has been established that a complex of several enzymes is involved in the complete hydrolysis of the polysaccharide. The released reducing sugar may be composed of saccharides of varying size. These reasons may account for the conflicting results that have been recorded for the interrelation between the enzymes responsible for the various changes in a particular substrate.

The present abstract will endeavour to summarize our present knowledge of the enzyme complexes hydrolyzing the glycosidic bonds in three polysaccharides which contain either hexosamines, hexuronic acids or both.

Chitinase. Karrer & Hofmann (1929) first made preparations from the intestinal canal of *Helix pomatia* which hydrolyzed fungus chitin to give 80 % of the theoretical yield of crystalline *N*-acetylglucosamine. Later Zechmeister, Tóth & Bálint (1938) succeeded in separating *β-d*-glucosidase, *α-d*-galactosidase and chitinase from emulsin preparations by filtration through a column of bauxite. In 1939 further observations were published on the fractionation of emulsin (Zechmeister & Tóth, 1939) and of glycerol extracts of the digestive tracts from *Helix pomatia* (Zechmeister, Tóth & Vajda, 1939). By the same adsorption technique the authors found that 44–83 % of the enzyme hydrolyzing chitodextrin was present in the filtrate. This enzyme however could not attack chitobiose. The filtrate contained only 0–25 % of the chitobiase originally present in the crude preparation of emulsin. The results using *Helix pomatia* preparations were substantially the same. The *β*-phenyl-*N*-acetylglucosaminidase present in the crude preparations accompanied the chitodextrinase during fractionation. From the results in these papers and the marked difference in the pH optimum of chitinase (pH 5·2) and the glucosaminidase (pH 3·6) (Neuberger & Pitt-Rivers, 1939) it does not seem likely that the latter enzyme is an active component of the chitinase complex, though further work is necessary to settle this point definitely. Zechmeister *et al.* (1939), however, have established that at least two enzymes are involved in the degradation of chitodextrin to *N*-acetylglucosamine.

Pectinase. Pectinase was first discovered in 1898 in barley malt. The enzyme produced 12 % of the theoretical amount of reducing sugar from pectin. This enzyme has since been shown to be formed by a variety of bacteria and fungi and to be present in various fruits. Its formation, however, has often been detected only by the disappearance of pectin added to culture media for micro-organisms. Waksman & Allen (1933) prepared cell-free extracts containing pectinase from a strain of *Penicillium* by H_2O extraction, followed by successive alcohol and acetone precipitations. The authors measured enzyme activity by the loss of precipitability of polygalacturonic acid by $Ca^{\cdot\cdot}$. They found that the pH optimum of the enzyme thus measured was less than 4·0. Kertesz (1936) found that the optimum pH of pectinase was 3·3–3·5; he used the change in viscosity of a 1 % pectin solution caused by a standard amount of enzyme in 30 min. at 30°. This value agrees with Davison & Willaman's (1927) determination, using the release of the reducing sugars to follow enzyme action. Most of these enzyme preparations appear to have contained pectin-esterase (pectase) which splits the methoxyl group from the esterified galacturonic acid. The recent findings by Jansen & MacDonnell (1945) may provide reasons for suspecting the results of studies made with these mixed enzyme preparations.

Jansen & MacDonnell (1945) preferentially inactivated the pectinesterase in pectinase preparations from orange flavedo by repeated treatment at pH 0·6 and 25°. The activity of the remaining 10 % of the original pectinase, possessing no pectinesterase activity, was then examined on pectinic acid preparations adjusted to varying methoxyl contents by either pectin-esterase or alkali. They found that the amount of the reducing sugar liberated by the enzyme acting at pH 4 for 100 hr. was inversely proportional to the methoxyl content of the polysaccharide. Further, the rate of action of pectinase on fully esterified polygalacturonic acid was so slow as to be negligible. Similar results were obtained when enzyme activity was measured by the time required to reduce the viscosity of the pectinic acids to 10 % of the original value. These results suggest that free carboxyl groups on the pectinic acid are necessary for enzyme action. The authors suggest, from other results, that adjacent —COOH groups are probably necessary for rupture of a glycosidic bond by pectinase. They also suggest that the initial rapid stage of hydrolysis of pectinic acids corresponds to the production of a disaccharide. This is hydrolyzed to galacturonic acid in the second very much slower stage.

Hyaluronidase. An enzyme which hydrolyzes the polysaccharide hyaluronic acid was first recognized by Meyer, Dubos & Smythe (1937) in pneumoccal cultures. Duran-Reynals (1928, 1929) and McClean (1930, 1931) had previously independently discovered that 'diffusing factors' were present in testicular extracts and in cultures of some bacteria (Duran-Reynals, 1933; McClean, 1936). Solutions containing these factors, injected intracutaneously, increased the rate of spread of inert substances in the dermis. Duthie & Chain (1939) and Chain & Duthie (1940) showed that hyaluronidase and spreading activities were probably due to the presence of the same substance and McClean (1943) has since shown that there is good correlation between hyaluronidase potency as measured by two different methods and the spreading factor activity of various preparations.

The antigenic specificity and different properties of hyaluronidases from various sources suggest the possibility that enzyme activity may be due to various hyaluronidases rather than to a single enzyme present in all sources. Recent results (Hahn, 1945; Rogers, 1945, 1946) have shown that the end-products of hydrolysis of hyaluronate differ according to the source of the enzyme. It is also claimed (Hahn, 1945) that crude testicular extracts contain two enzymes, one of which hydrolyses hyaluronate to oligosaccharides, the second completes the hydrolysis to monosaccharides.

When the amount of *N*-acetylglucosamine released by enzymic hydrolysis of hyaluronate is measured by the Morgan & Elson (1934) test, its amount is very much greater than can be accounted for by either the amount of reducing sugar liberated or sometimes even by the total hexosamine content of the polysaccharide (Meyer, Chaffee, Hobby & Dawson, 1941). Recent results (Rogers, 1946) have shown that after partial hydrolysis of hyaluronate by testicular enzyme about 50 % of the apparent *N*-acetylglucosamine is still attached to units not diffusible through cellophan. On treatment at 100° in the presence of N-CH_3COOH the amount of colour given in the Morgan & Elson (1934) test, by the diffusible substances present after hydrolysis of the polysaccharide by hyaluronidase from streptococci and *Cl. welchii*, rapidly falls. The conditions of the acid treatment were such that no de-acetylation of *N*-acetylglucosamine would occur. The results suggest that the amino sugar released from hyaluronate by enzymic hydrolysis may be a labile substance which is easily changed into *N*-acetylglucosamine by treatments with acids; further work is necessary to verify this.

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Metabolic Products containing Uronic Acids. By G. F. MARRIAN

Conjugation with glucuronic acid has for long been regarded as one of the principal 'detoxication' mechanisms by which the animal body is enabled to decrease the toxicities of certain toxic substances absorbed from the gastro-intestinal tract as a preliminary to their elimination in the urine. Until recently the substances which were known to be 'detoxicated' in this way could be divided into two distinct groups—one group, comprising phenol, *p*-cresol, and indoxyl, being what may be termed 'physiologically normal toxins' inasmuch as they may normally be produced in considerable amounts in the intestinal tract from amino acids by bacterial action; the other group containing substances like menthol, borneol, phenolphthalein, diethylstilboestrol, *p*-hydroxybenzenesulphonamide, etc., etc., which are not normally in the animal dietary and which are not produced in the intestinal tract from normal foodstuffs.

It is now known that in addition to these two groups of exogenous toxins several normal metabolites of endogenous origin may undergo conjugation with glucuronic acid in the animal body. There are good grounds therefore for considering conjugation with glucuronic acid as a normal metabolic process rather than as a 'detoxication' mechanism.

The only normal metabolites which are known to be conjugated with glucuronic acid in the body are steroids. Among the steroids which are believed to occur in urine (human) as glucuronides are the following: oestriol, pregnane-3(α), 20-diol, pregnane-3(α)-ol-20-one, and pregnane-3(α), 17, 20-triol.

Oestriol 'monoglucuronide' was isolated in 1934 from human pregnancy urine by Cohen & Marrian. Subsequently it was shown that the uronic acid residue is linked to the oestriol through the C_{16} or C_{17} hydroxyl group of the latter. The uronic acid component of this 'glucuronide' has not yet been identified with certainty as glucuronic acid.

Pregnane-3(α), 20-diol glucuronide was isolated as a crystalline sodium salt from human pregnancy urine by Venning & Browne in 1936. Subsequent work by these authors clearly showed that this compound is the main end-product of progesterone metabolism in women during pregnancy and in the menstrual cycle. Recently Heard, Hoffman & Mack (1944) have shown that the uronic acid is linked in this compound to the C_3 hydroxyl group of pregnanediol, while Heubner, Overman & Link (1944) have shown by their synthetic experiments that the natural product is identical with pregnane-3(α), 20-diol-3-β-*d*-glucuronide.

Marrian & Gough (1946) have shown that sodium pregnanediol glucuronidate prepared

from human pregnancy urine contains about 15–20 % of a water-soluble derivative of pregnane-3(α)-ol-20-one as an impurity. This substance, which is believed to be sodium pregnane-3(α)-ol-20-one glucuronidate, but which has not yet been isolated, cannot be removed by the usual methods of purification.

What appears to be a glucuronide of a not yet identified ketosteroid has been isolated from the urine of a girl with virilism symptoms by Strickler, Shaffer, Wilson & Strickler (1943), while more recently Mason & Kepler (1945) have put forward evidence that the pregnane-3(α),17,20-triol, first isolated by Butler & Marrian (1937, 1938) from the urine of women with adrenal hyperplasia, occurs also as the glucuronide. It is noteworthy that the sodium salt of this triol glucuronide cannot be separated from sodium pregnanediol glucuronidate by the usual purification procedures.

In view of the important role of glucuronic acid conjugation in 'detoxication' and in steroid metabolism, the widespread occurrence in animal tissues of an enzyme specific for the hydrolysis of β-glucuronides is of considerable interest. This 'β-glucuronidase' was first demonstrated in animal tissues by Masamune (1934) and has since been studied extensively by Oshima (1934, 1936), Fishman (1939, 1940) and Graham (1946). The biochemical role of this enzyme is still very obscure. It has been supposed by Fishman that it functions in the body synthetically by catalyzing the conjugation of hydroxy compounds with glucuronic acid. This theory was based on experiments in which increases in the glucuronidase activities in certain tissues were observed subsequent to the administration to the animal of substances such as borneol and menthol, the supposition being made that the increase in enzyme is a response to the flooding of the body with the substrate for its synthetic action. This theory, attractive though it is, does not seem to be altogether compatible with certain findings of Lipschitz & Bueding (1939). The latter workers have shown that surviving liver and kidney slices will synthesize conjugated glucuronides in the presence of glucuronidogenic substances, and they have shown that while the rate of glucuronide synthesis can be increased by the addition of dihydroxyacetone, lactic and pyruvic acids, it cannot be increased significantly by the addition of glucuronic acid itself. These findings suggest that glucuronide synthesis cannot be a simple enzyme catalyzed reaction between glucuronic acid and the aglucone as is supposed by Fishman. Lipschitz & Bueding suggest that the first step in glucuronide synthesis may be combination between the aglucone and triose, and they believe that glucuronidogenesis and carbohydrate synthesis from three-carbon compounds may be competing processes. Further investigations on the mechanism of glucuronide synthesis and on the biochemical role of β-glucuronidase would be most desirable.

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PROCEEDINGS OF THE BIOCHEMICAL SOCIETY

The **Annual General Meeting** *of the Biochemical Society was held in King's College, Strand, London, W.C. 2, on Saturday, 16 March 1946 at 2.15 p.m., when the following papers were read:*

The Capsular Substance of *Bacillus anthracis*. By W. E. Hanby and H. N. Rydon

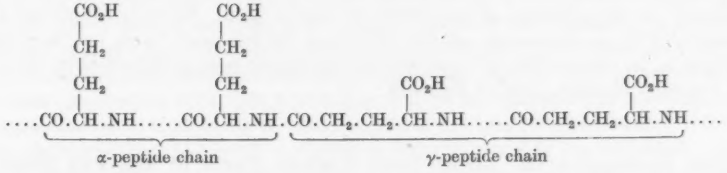
Ivanovics and his colleagues (1937, 1938) isolated the capsular substance of *B. anthracis* and related organisms and showed it to be a polypeptide of molecular weight about 6000, built up, to the extent of at least 86 %, of *d*-(−)-glutamic acid residues (Bovarnick, 1942). The present paper is concerned with the further investigation of this substance.

An improved extraction procedure, based on the observation (due to Dr P. Bruce White, F.R.S.) that the capsular substance is soluble in acid, but insoluble in slightly alkaline 75 % alcohol, has led to the isolation of the capsular substance from two strains of *B. anthracis* as a polypeptide of much higher molecular weight than that obtained by the earlier workers; our least degraded material had a minimum molecular weight of 53,000, and is thus properly regarded as a protein of particularly simple composition. The high viscosity of its aqueous solutions indicates that the molecule is threadlike rather than globular. All our preparations, of minimal molecular weights varying from 4500 to 53,000, have haptenic properties, giving precipitin reactions with anticapsular serum down to dilutions of 1 in 2×10^7.

It is an easy matter to isolate large amounts of *d*-(−)-glutamic acid from hydrolysates of the capsular polypeptide (Brückner & Ivanovics, 1937) and we have shown, by a new method involving conversion of the hydrolysate glutamic acid into pyrrolidone-carboxylic acid followed by partition chromatography (Martin & Synge, 1941; Gordon, Martin & Synge, 1943; Liddell & Rydon, 1944), that at least 99 % of the nitrogen of the polypeptide is present in glutamic acid residues; we were unable to detect any traces of other amino-acids, neither was there any significant amount of amide-N.

The behaviour of the capsular substance with nitrous acid indicates the presence of γ-peptide linkages, and this has been confirmed by electrometric titration, which reveals free α- and γ-carboxyl groups. From the titration results we have calculated that our least degraded material contains one free α-carboxyl to about ten free γ-carboxyls; the proportion of free α-carboxyls is less in the more degraded materials.

It is concluded from these results that the capsular substance has a structure of the type:



in which all the glutamic acid residues have the 'unnatural' *d*-configuration. The capsular substance is not actively antigenic and can only be made so with difficulty by coupling with proteins; its biological function is probably to protect the organism in virtue of its resistance to the ordinary proteolytic enzymes.

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The Role of Linoleic Acid, α-Tocopherol and other Fat-soluble Substances in the Nutrition of an Insect (*Ephestia kuehniella* Lep.). By G. FRAENKEL and M. BLEWETT

The caterpillars of the flour moth, *Ephestia kuehniella*, grow well on a diet consisting of casein (20), glucose (80), yeast (5), salts (2), water (15) and wheat-germ oil (1). Without wheat-germ oil growth is slow, the mortality high and the moths never emerge from the pupae. With suboptimal doses moths emerge with the wings partly or entirely lacking in scales. Graded doses of the oil produce a graded response with regard to growth rate and state of scales.

With the saponifiable fraction of wheat-germ oil alone growth is slow, but the moths emerge with perfect wings. With the unsaponifiable fraction alone growth is fast, but moths never emerge. The active substance in the saponifiable fraction is linoleic acid. Graded doses of linoleic acid give rise to moths exhibiting graded degrees of scale deficiency. Linolenic acid has approximately the same qualitative and quantitative effect as linoleic acid. Oleic acid has no effect.

The chief active substance in the unsaponifiable fraction is vitamin E (α-tocopherol) and there are possibly other substances. One of the effects of the unsaponifiable factor(s) is that of an antioxidant which stabilizes linoleic acid. In the absence of it pure linoleic acid is ineffective. The unsaponifiable factor(s) can be replaced by ethyl or propyl gallate and by ascorbic acid. Apart from this stabilizing action on linoleic acid, the unsaponifiable fraction has an independent and pronounced growth effect. A slight growth effect has also been obtained with α-tocopherol alone, but not with gallates or ascorbic acid alone.

The effect of a number of natural fats on scales and emergence is strictly parallel to their linoleic acid content. No emergence takes place with coconut, allenblackia, cod-liver oil or halibut-liver oil (little or no linoleic acid), while lard and butter (*c.* 5 % linoleic acid) have a slight positive effect on scales. Growth, on the other hand, is fast with cod-liver oil, halibut-liver oil, lard or butter. Docosahexenoic acid from cod-liver oil has a very favourable effect on growth, but none on scales.

Large quantities of cereal starches in the diet have a favourable effect on scales which is attributed to the presence of impurities of linoleic acid. No such effects have been encountered with potato starch which is free from linoleic acid. There are very striking similarities in the effect of linoleic acid and natural oils on rats, as described by G. O. Burr and I. Smedley-Maclean, and on *Ephestia*.

Penicillin Production on Juices from Various Parts of the Pea Plant. By R. P. COOK and MARGARET B. BROWN

The juice from green seed peas in pod forms a good basis for a medium for the production of penicillin (Cook & Tulloch, 1945). An investigation was carried out using juices expressed from parts of the pea plant which would form waste in canning for their peni-

cillin-producing activities. Average values for the yield of juice and the chemical composition from parts of the pea plant are given in the table. The high yield of juice and its relatively high dry-matter concentration are noteworthy. To a basal medium (Cook, Tulloch, Brown & Brodie, 1945) the juices were added. Growth was very good with all juices. The maximum yields of penicillin obtained with *P. notatum* 1249 B21 were: haulm juice approx. 30 units/ml., pod juice approx. 70 units/ml., mixed haulm and pod juices approx. 70 units/ml. The juice from peas in pod of Kelvedon Wonder and Laxton's Progress varieties gave approx. 175 units/ml., but the juice from the large-podded 'V/C' variety gave only approx. 100 units/ml.

		Juice expressed						
	As % of entire plant	As % of entire plant	As % of part	Dry matter (g./100 ml.)	Ash (g./100 ml.)	Total N (g./100 ml.)	Carbohydrate (as glucose) after hydrolysis (g./100 ml.)	Fibrous residue as %-of as % of entire plant
Haulms	40	21	54	12·9	1·6	0·32	6·4	18
Pods	40	27	69	9·2	0·5	0·32	7·4	8
Haulm and pods	80	48	60	10·9	1·0	0·32	6·9	26
Green seed peas in pod	60	—	55	5·2	0·35	0·45	2·9	—

It is suggested that juices made from the waste pods and/or haulms might be of value as a stock fodder. They are free from indigestible fibre (Woodman & Evans, 1944).

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Further Studies of the Van den Bergh Reaction in Haemolytic Jaundice. By C. H. Gray and Joanna Whidborne

A modification of the Rappaport & Eichhorn (1943) method for determination of direct and indirect bilirubin has made it possible to plot curves representing the relationship between azobilirubin production and time. Results of such investigations on sera from patients with acute hepatitis and obstructive jaundice have already been described (Gray & Whidborne, 1945, 1946). Preliminary results then suggested that the form of curve given by sera from patients with haemolytic jaundice differed from that given by sera from patients with other forms of jaundice. Of 57 sera from patients with haemolytic jaundice, the majority gave retarded curves similar to those previously described, but a few of the curves were of the type seen in acute hepatitis and obstructive jaundice, and others gave curves intermediate between these two extremes. Sera from cases of haemolytic jaundice following incompatible blood transfusion at first showed retarded curves of the usual haemolytic type, but within 2 or 3 days the shape of the curve changed so as to approximate to that found in acute hepatitis and obstructive jaundice.

It is considered that the great variation in form of curve is less consistent with the existence of two forms of bilirubin than with an acceleration of azobilirubin production by some factor present in the serum in all cases of acute hepatitis and obstructive jaundice and in some cases of haemolytic jaundice. The behaviour of haemorrhagic pleural effusions and other such fluids in giving a similar range of reactions rules out the liver as necessarily playing any direct part in altering the nature of the reaction.

The curves of azobilirubin production under the conditions in which we studied the direct reaction are not exactly comparable with the ordinary direct Van den Bergh reaction, since the presence of urea in the direct buffer increases the extent of the direct reaction and accelerates the rate of azobilirubin production. This increase amounts only to about 5 % in sera from cases of acute hepatitis and obstructive jaundice and is therefore insufficient to affect the earlier interpretation of our results. On the other hand, with sera from patients with haemolytic jaundice the addition of urea profoundly alters the course of the direct reaction and results thus obtained cannot be interpreted as representing the ordinary Van den Bergh reaction.

It is evident that the proportion of so-called 'direct bilirubin' is affected by the presence or absence of urea as well as by the pH of the reaction mixture. It is suggested that the terms 'direct' and 'indirect' bilirubin be abandoned in favour of 'direct-indirect quotient', since there is no conclusive evidence that two forms of bilirubin exist.

The 'direct-indirect quotient' (D.I.Q.) is defined as follows:

$$\frac{\text{Final amount of azobilirubin formed in the direct reaction}}{\text{Total amount of azobilirubin formed in the indirect reaction}} \times 100.$$

The D.I.Q. as determined by the Malloy & Evelyn (1937) method provides a satisfactory means of distinguishing between haemolytic and other forms of jaundice.

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The Metabolism of Marfanil (Homosulphanilamide) in the Rabbit. By R. L. HARTLES and R. T. WILLIAMS

Blaschko & Duthie (1945) have shown that Marfanil (*p*-sulphonamidobenzylamine hydrochloride) is a substrate for amine oxidase (cf. Beyer & Govier, 1945). This suggests that in the body it would undergo a deamination to form *p*-sulphonamidobenzaldehyde which would then be oxidized to *p*-sulphonamidobenzoic acid. The overall reaction would be as follows:

Marfanil was therefore fed to rabbits and their urine examined for this acid. On acidifying 'Marfanil urine' with HCl, a copious crystalline precipitate was obtained. This precipitate was collected and recrystallized from aqueous alcohol, and the purified product was identified as *p*-sulphonamidobenzoic acid (m.p. 295°) identical with an authentic sample. About 50 % of the Marfanil fed (2 g.) can be recovered in 24 hr. by acid precipitation as *p*-sulphonamidobenzoic acid.

Besides undergoing oxidation in the side-chain, Marfanil also suffers, to a small extent, nuclear oxidation to a phenolic substance, for about 7 % of the drug fed is excreted as an ethereal sulphate. This second oxidation could be the result of either direct nuclear oxidation of Marfanil before deamination or further oxidation of *p*-sulphonamidobenzoic acid. The latter alternative was eliminated by the finding that the feeding of *p*-sulphonamidobenzoic acid does not cause an increased output of ethereal sulphate.

It appears, therefore, that in the rabbit Marfanil undergoes two types of oxidation, the major one being rapid oxidation of the side chain, the nuclear oxidation being only a very minor reaction.

It is likely that *p*-sulphonamidobenzoic acid has no antibacterial activity. The rapid transformation of Marfanil into this acid *in vivo* would therefore explain the systemic inactivity of the Marfanil molecule.

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Some Observations on the Composition of Defatted Fowl Plasma. By C. Waymouth and J. N. Davidson

When fowl plasma is treated in the cold with ethanol and ether to remove lipid materials without denaturing the proteins (Waymouth & Davidson, 1945), and the protein powder redissolved to the original volume, the resulting fluid retains many of the physical characteristics of the original plasma and has therefore been found suitable for use in a tissue culture medium. It is to be expected from the method of preparation that those constituents of plasma which are insoluble in about 60 % alcohol would be absent from the reconstituted fluid. To examine whether inorganic salts, in particular, were removed from the plasma during the defatting procedure, the chloride content of the defatted fluid was determined. In no case was more than 7 % of the chloride of the plasma found in the extracted material. The acid-soluble phosphorus of the extracted plasma, and the inorganic phosphorus which forms the greater part of this fraction in fowl plasma, were retained to the extent of 50–90 % of the amounts in the untreated plasma. Lipid-P and residual-P were greatly reduced. The reconstituted protein solution also contained a large proportion of the plasma calcium. It is known that a high proportion of plasma calcium may be in the unionized form. Grollman (1927), Laskowski (1933) and Greenberg, Larson, Pearson & Burmester (1936) showed that in the fowl, in contrast to most animals, a considerable fraction of the inorganic phosphate is normally in a non-diffusible condition. It is suggested, in agreement with the hypothesis of Grollman, that the non-diffusible calcium phosphate is in a very loose combination with protein, that in fowl plasma the greater part of both the calcium and the inorganic phosphate may normally be associated with native protein, and that the associations are not broken by the treatment of the plasma which dissociates the lipoprotein complexes.

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Histidine Deficiency in Rats. By A. Neuberger and T. A. Webster

Observations on a Case of Alkaptonuria. By A. Neuberger, C. Rimington and J. M. G. Wilson

Serum Albumin and Globulin Levels in Premature Infants. By C. RIMINGTON and J. A. BICKFORD

Despite the increased interest in this subject which has been displayed during the last few years, there is still very little data from which to compare the blood serum protein levels of the human infant and mother at the time of birth and only the most meagre information concerning the values of these constituents in premature human infants or during foetal development. Knowledge of the biogenesis of serum albumin and globulin is therefore largely conjectural.

The subject has not only considerable scientific interest but is also of practical importance, bearing, as it does, upon the problems of immunity and susceptibility to neonatal infection. The calf is born with a very low serum globulin level but as a result of intake of cow's colostrum, the globulin very rapidly rises (Howe, 1921; Orcutt & Howe, 1922). Should colostrum be withheld, the globulin level remains low and such animals readily contract colon bacillus septicaemia which may prove fatal (Famulener, 1912). In the case of the human infant and mother, the placental system is relatively more simple and a passive immunity of limited duration to certain diseases such as measles, scarlet fever and diphtheria may be conferred by the mother, nevertheless susceptibility to pyogenic infections, coli infections etc., persists and the immunological response even of the full term new-born infant is but poorly developed. McKhann & Kapnick (1938) have said in this connexion: 'One should not be satisfied by the term "serologic immaturity" but must attempt to establish by chemical and physiological studies the basis of such immaturity. Is the genesis of plasma proteins the important factor?'

The present investigation has attempted to fill a gap in our knowledge concerning the synthesis of plasma proteins in the developing infant and so provide a basis for any arguments concerning the possible relations of susceptibility to neonatal infections and the degree of development of the protein-elaborating mechanisms. Blood samples were taken from the mothers by venepuncture, usually at the commencement of labour, and after delivery from the placental end of the cord by pouring. The serum was separated, centrifuged, and the analyses carried out with as little delay as possible. The micro-Kjeldahl method with distillation and titration of the ammonia was used exclusively, the albumin and globulin being separated by the sodium sulphite procedure and selenium oxide being used as the catalyst during incineration for a standardized period of time. Our technique also provided for a determination of N.P.N. as follows:

(1) *Total protein*+N.P.N.: 0·1 ml. serum used for digestion etc.

(2) *Total protein*: 0·1 ml. serum+2·4 ml. H_2O+2·5 ml. of 10% (w/v) trichloroacetic acid, centrifuged and ppt. dissolved in 0·5 ml. of 2N-NaOH and transferred with water washings to a digestion flask. (1)–(2)=N.P.N.

(3) Albumin: 0·1 ml. serum+1·9 ml. of 42% (w/v) cryst. Na_2SO_3 left for 0·5 hr., filtered and 1 ml. of filtrate used for N determination. Value corrected for N.P.N. and globulin calculated by difference.

The values for albumin and globulin and total protein were checked frequently by comparison with values obtained in another laboratory using the ammonium sulphate and biuret colorimetric methods.

The cases were as far as possible normal pregnancies except that delivery occurred before the calculated date. The very early foetal specimens (20 weeks) were obtained from cases in which pregnancy had to be terminated on account of pulmonary tuberculosis in the mother.

Analysis of results. Complete sets of figures were obtained on 38 cases. Attention has been directed chiefly towards possible relationships between the following:

(1) Maternal and infant albumin or globulin respectively.

(2) Maternal albumin and globulin and duration of pregnancy.

(3) Infant albumin and age of foetus.

(4) Infant globulin and age of foetus.

Our figures have been subjected to a rigid statistical analysis (for assistance with which we are greatly indebted to Dr C. C. Spicer of the British Bombing Survey Unit) and the following points have emerged. Multiple regressions calculated on duration of maternal and foetal albumin and maternal and foetal globulin respectively show that:

(1) The regressions of the maternal proteins are not significant in either case.

(2) Regression of both foetal proteins on length of gestation is significant and not improved by eliminating the variability of the corresponding maternal protein levels. The foetal regression equations are

$$D = 27{\cdot}2709 + 4{\cdot}0705\ \mathrm{G},$$

$$D = 20{\cdot}5301 + 3{\cdot}8814\ \mathrm{A}.$$

A further regression of foetal albumin and globulin on duration allowed a test to be made of the significance of the difference between the rates of increase of the two proteins. The equation is

$$D = 18{\cdot}8794 + 3{\cdot}1097\ \mathrm{G} + 2{\cdot}7206\ \mathrm{A}.$$

The regression coefficients are both highly significant but the difference between them of 0·3891 with standard error 1·4633 is clearly not significant.

In figures illustrating the findings, the calculated lines of best fit have been drawn through the experimentally recorded points and it is clear that their slopes are almost identical or in other words, assuming that the increase of either protein during development from the 20th to 40th week may be linearly represented, both albumin and globulin are increasing at the same rate. Calculation of the customary A/G ratio leads here to a completely false impression, this ratio being at 20 and 40 weeks, 4·47 and 1·73 respectively.

Many interesting points arise out of these findings, for example, whether albumin and globulin synthesis are locally and chemically as well as temporarily related in the foetus: what the osmotic conditions will be prevailing between foetal blood and tissues when the albumin is at very low levels and also whether a premature baby starting life—with its attendant risk of infection—will become immunologically mature at an age determined by the date of its conception or of its birth.

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PROCEEDINGS OF THE BIOCHEMICAL SOCIETY

The **250th Meeting** *of the Biochemical Society was held in the Department of Biochemistry, University Museum, Oxford, on Saturday, 4 May* 1946 *at* 1.30 *p.m., when the following papers were read:*

COMMUNICATIONS

The Specific Property Solubility Test for Protein Purity and its Complete Analysis at Constant pH and Temperature. By J. S. Falconer and D. B. Taylor

The Preparation of Pure Liver Esterase. By J. S. Falconer and D. B. Taylor

The Metabolism of Aromatic Amines. By L. A. Elson, F. Goulden and F. L. Warren

In connexion with work on the metabolism of carcinogenic azo compounds it was found necessary to study the fate in the body of a number of aromatic amines which may be formed by fission of the azo compounds themselves. The amines were administered to rats by intraperitoneal injection, and any changes in the daily excretion of ethereal sulphate, glucuronic acid, neutral sulphur, and inorganic sulphate in the urine determined as described previously for aromatic hydrocarbons (Elson, Goulden & Warren, 1945). At the same time estimations of free and acetylated amines in the urine were carried out by means of the colour given on diazotization and coupling with *N*-sulphatoethyl-*m*-toluidine (cf. Elson & Warren, 1944). In many cases metabolic products present in too small an amount for isolation could be characterized by means of the absorption curve of this colour determined by the use of a series of colour filters with the Hilger 'Spekker' photoelectric absorptiometer. Some of the results obtained are given in the table on p. xxx.

Aniline at a dosage of 72·5 mg./kg. is excreted exclusively as ethereal sulphate, whereas the equivalent dose of *p*-amino-phenol (90 mg./kg.) is excreted entirely as glucuronate. This appears to preclude the long-held view that aniline is converted in the body to *p*-amino-phenol and this then excreted as a conjugate with sulphate or glucuronate, and suggests that ethereal sulphate formation can take place directly in the aromatic nucleus without preliminary formation of free phenolic compounds.

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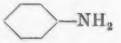
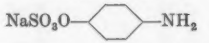
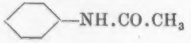
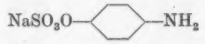
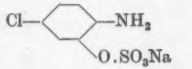
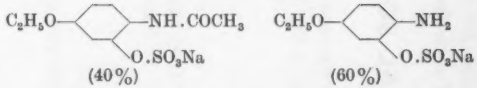
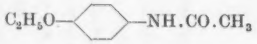
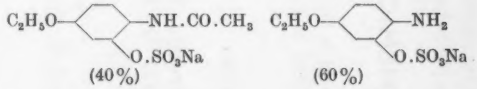
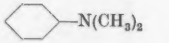
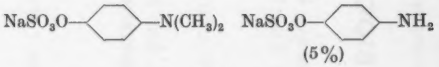
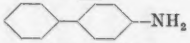
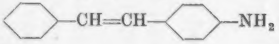
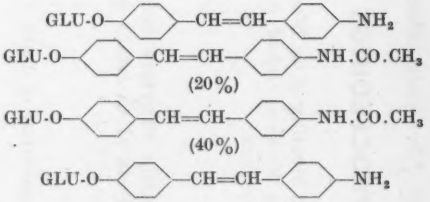
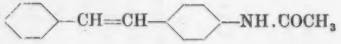
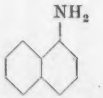
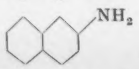
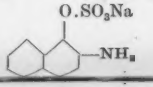
The Origin and Function of Seminal Fructose. By T. Mann

The chief reducing and yeast-fermentable sugar of the seminal plasma is *d*(−)-fructose as shown by the purification of the sugar and the isolation of crystalline methylphenylfructosazone (Mann, 1946). Very little glucose, if any, is present; this was shown by using mould glucose oxidase which selectively oxidizes glucose leaving fructose untouched. The level of fructose in the seminal plasma fluctuates from one species to another, and even within the same species there are individual differences. The values for seminal fructose range from a few mg. % in boar to several hundred mg. % in bull, ram, rabbit and man;

Substance	Dose (mg./100 g. rat)	Neutral sulphur	Ethereal sulphate	Glucuronic acid	Acetylation	Probable nature of principal metabolic products
	7·25	–	+	–	3·5% as acetanilide, 0·7% as aniline	
	14·0	Slight	+ +	Slight		
	9	–	+	Slight	Almost completely deacetylated	
	20	–	+ +	+ +		
	40	–	+ +	+ +		
	7·8	–	+	–	0·7% as free and acetylated *p*-chloraniline	
	20	.	+ +	–	.	
	20	.	+ +	–	0·35% as phenacetin, 0·2% as phenetidine	
	17	Slight	+ +	Slight	About 5% demethylated, no acetylation	
	9·25	–	–	+	No acetylation	
	16	+	+	+ +		
	8·6	.	–	+ +	.	
	14·6	.	+	+ +		
	8·5	.	–	+ +	.	
	19	+ +	–	+	.	
	14·7	.	–	+	.	
	18·7	+	+	+ +	.	
	17·4	+	+ +	Trace	.	

in bull the concentration may exceed 1000 mg. %. In all instances there is a close agreement between the values for fructose and for yeast-fermentable sugar. On the other hand, in almost every case the seminal plasma contains some reducing but non-fermentable substances.

Spermatozoa obtained directly from the epididymis contain hardly any fructose at all. They acquire the sugar from the accessory glands of reproduction during their passage through the male generative tract. The seminal vesicles are the main source of fructose. In the rabbit, fructose is found mainly in the gl. vesicularis of the prostate gland. The function of fructose in the seminal plasma is to supply a readily fermentable substrate for the glycolytic system within the spermatozoa. The fructolysis in sperm begins with a hexokinase reaction between fructose and adenosinetriphosphate preformed in the cells. As a result monophosphohexose is formed. From here the breakdown leads through diphosphohexose, phosphotriose, phosphoglyceric and pyruvic to lactic acid, and in this respect the process in whole semen closely resembles reactions in washed spermatozoa to which sugar was added artificially (Mann, 1945).

Under natural conditions spermatozoa utilize fructose available to them in the seminal plasma. But their enzymic equipment enables them to glycolyze glucose and mannose equally well. This explains why it was possible in the past to employ successfully glucose as the nutrient component of artificial media for storage of mammalian semen.

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The Formation of Nicotinamide from Nicotinic Acid by Rat Tissues. By P. ELLINGER

Nicotinic acid and nicotinamide are metabolized by certain animals into nicotinamide methochloride. Perlzweig, Bernheim & Bernheim (1943) show that methylation of nicotinamide *in vitro* occurred in liver, and nicotinamide but not nicotinic acid was methylated. Ellinger & Coulson (1944) observed in man that in the formation of nicotinamide methochloride from nicotinic acid two separate processes are involved.

To study the formation of nicotinamide from nicotinic acid a method similar to that of Perlzweig *et al.* (1943) was used. Pieces of various organs of freshly killed rats were weighed and cut up into slices of 0·2–0·4 mm. These were shaken together with liver slices in Krebs (1932)-Ringer aerated with a O_2-CO_2 mixture, together with an NH_4 nicotinate solution at 37° for 2–3 hr.; in some instances the organ slices were shaken with the NH_4-nicotinate separately, and then the supernatant liquid added to the liver slices and again shaken. The liquid was decanted from the tissue, protein removed by precipitation with trichloracetic acid, heating to 70–80° and centrifuging. The clear liquid was used for the estimation of formed nicotinamide methochloride, by the method of Perlzweig *et al.* (1943) and that of Coulson, Ellinger & Holden (1944). The former gave frequently too high values, since the carbinols to be measured were immediately oxidized into pyridones having a higher fluorescence efficiency. Controls of the methylating efficiency of the liver used were made by adding nicotinamide to a sample and blanks without nicotinate were run, their values being subtracted from those of the nicotinate values. In a number of cases glutamine or asparagine was added to the system liver-nicotinate. In some instances the methylating efficiency of liver of rats was tested whose previous nicotinamide methochloride elimination was known. Methionine was also added in some cases. The results are given in Table 1.

Table 1. *Synthesis of nicotinamide methochloride*

Tissue	No. of experiments	Limits of weight of liver (mg.)	Substrate	Limits of weight of tissue in substrate (mg.)	μg. nicotinamide methochloride formed per g. liver (average in brackets)
Liver	16	230–610	Nicotinamide, 1 mg.	—	6–35 (18)
Liver	10	290–580	Nicotinate, 1 mg.	—	0–3
Kidney	4	350–420	Nicotinate, 1 mg.	230–320	0–2
Kidney	4	330–490	Nicotinate, 1 mg.	220–340	0–1
Brain	4	380–520	Nicotinamide, 1 mg.	180–280	0–2
Brain	4	370–610	Nicotinate, 1 mg.	170–300	0–1
Liver	4	440–560	Kidney; nicotinamide, 1 mg.	140–590	14–28 (21)
„	16	340–590	Kidney; nicotinate, 1 mg.	255–585	12–33 (17)
„	4	360–440	Brain; nicotinamide, 1 mg.	130–190	8–18 (14)
„	10	380–520	Brain; nicotinate, 1 mg.	120–210	7–29 (21)
„	4	330–430	Spleen; nicotinate, 1 mg.	165–225	0
„	4	320–480	Motor muscle; nicotinate, 1 mg.	530–620	0
„	4	330–450	Heart muscle; nicotinate, 1 mg.	260–300	0
„	4	350–450	Pancreas; nicotinate, 1 mg.	50–70	0–1
„	4	380–510	Intestine mucosa; nicotinate, 1 mg.	110–130	0–1
„	8	360–580	Kidney; nicotinate, 1 mg., anaerobic	330–470	0–1
„	8	420–620	Nicotinate, 1 mg.; glutamine, 10 mg.	—	0–16 (7)
„	4	390–580	Nicotinate, 1 mg.; asparagine, 10 mg.	—	0

Of the tissues examined kidney and brain alone were able to form the amide from ammonium nicotinate as in Krebs's (1935) experiments on glutamine. Glutamine added to liver and nicotinate produced in five or eight instances nicotinamide methochloride, whilst asparagine was inefficient in this respect. Kidney did not form amide from nicotinic acid anaerobically. Livers from rats with low nicotinamide methochloride elimination (20–40 μg./day) formed less nicotinamide methochloride (6–8 μg./g.) from 1 mg. nicotinamide than those from rats with high elimination (550–700 μg./day and 25–29 μg./g. respectively). Methionine added increased the nicotinamide methochloride formation from nicotinamide in livers with previously low yield.

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The Influence of Changes in Diet and of Drugs on the Urinary Elimination of Nicotinamide Methochloride by Rats. By P. ELLINGER

Rats of 250–400 g. receiving daily rations of 13 g. of a mixture of wheat and oats, 40 parts; extracted liver residue, 35 parts; wheat germ, 10 parts; dried yeast, 3 parts; salt mixture, 5 parts; peanut oil, 6 parts; cod-liver oil, 1 part; and 16 g. bread moistened with 16·5 ml. 10% dried milk in water were examined for their daily 24 hr. urinary elimination of nicotinamide methochloride by the method of Coulson, Ellinger & Holden (1944). This was fairly constant in the same rat if kept under constant conditions, but varied widely (20–1000 μg.) individually, although the rats received the same food and lived in the same room.

Changes in diet were made or drugs were administered which could be expected to influence the intestinal flora and subsequently the elimination of nicotinamide methochloride.

Omission of milk or adding of lactose (Rettger & Chaplin, 1921) (four experiments each) had no significant influence on the nicotinamide methochloride output.

When grain mixture and bread were replaced by equal weight of maize in four rats previously fed without milk, the nicotinamide methochloride output fell to 10·5, 11·2, 14·4 and 42·5% respectively of the predosing level; adding of milk increased the output in one of two cases by 33%. Krehl, Sarma, Teply & Elvehjem (1946) observed no changes when feeding maize to mature rats. Whether the maize affects the intestinal flora or releases a 3-acetyl-pyridine-like substance (Woolley, 1945, 1946) has to be ascertained.

10 g. of charcoal (activated B.D.H.), absorbing nicotinamide very little, added to the food reduced within 3 days the nicotinamide methochloride elimination to 6·4, 12·1 and 17·2 and 20·6% respectively of the predosing level. This was reached again 7 days after discontinuation of the charcoal.

30 mg. of sodium barbitone given daily with the food reduced the nicotinamide methochloride elimination to 20·2 and 31·0% respectively of predosing level; 40 and 60 mg. had no larger effect. Spontaneous recovery to about 60–75% of predosing level occurred while the drug was still administered. Subcutaneous doses of 60 mg. daily did not influence the elimination at all.

The effect of succinyl- (5% of food) or phthalyl-sulphathiazole (1–2% of food) varied considerably. With the former the nicotinamide methochloride elimination was reduced in twelve experiments to 11·1 to 96% of predosing level. With phthalyl sulphathiazole the corresponding values were 20·5 and 98% respectively.

In many cases symptoms of B_2 complex deficiencies developed beginning with loss of hair. Examination of changes in the bacteriological composition of faeces and calcium content, carried out in collaboration with H. S. Schutze and A. D. Emanuelowa, gave no conclusive results owing to spontaneous daily variations observed already by Topley & Fielden (1922).

To test the methylating mechanism of the liver 0·2 ml. of a mixture of equal parts of chloroform and carbon tetrachloride were injected intraperitoneally into four rats which had been previously tested for their response to 20 mg. of extra nicotinamide. Each injection increased the nicotinamide methochloride elimination to 150–300% of predosing level for 1 day and fell then slowly down to about 60% to rise again after a further injection; the response to nicotinamide dosage fell, however, to about 36–40% already after the first injection. The results confirm those of Perlzweig, Huff & Rosen (1946); they should not be explained by a reduced destruction but by a temporarily increased formation of nicotinamide methochloride.

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Penicillin Production on Fractions from the Pea (*Pisum sativum*). By R. P. Cook and Margaret B. Brown

By means of ethanol precipitation fractions may be made from aqueous extracts of ground dried peas or the juice from green peas in pod which support the growth of *Penicillium notatum*. At a concentration of 80 % (v/v) ethanol from either of these sources a fraction is precipitated which on redissolving in water is active in stimulating penicillin production. It was at first thought that the addition of the alcohol-soluble material increased the yield of penicillin, but it has now been found that the ethanol-precipitable water-soluble fraction alone at the proper dry-matter concentration gives high yields.

Surface cultures of *P. notatum* 1249 B21 have been used and a basal medium as described (Cook, Tulloch, Brown & Brodie, 1945). The yield of penicillin is proportional to the dry-matter concentration of the fraction reaching a maximum yield of at least 200 units/ml. at a value of 2·0 g./100 ml. of medium. The addition of increasing amounts of the alcohol-soluble fraction to a constant amount of the alcohol-precipitable fraction while resulting in a marked increase of growth of the mould lowers and finally inhibits penicillin production.

The ethanol-precipitable fraction is complex in nature containing mineral matter, N-containing compounds and complex polysaccharides. Attempts at a synthetic medium based on some of its known constituents have not as yet been successful.

The salts of the basal medium play an important role in penicillin production. The addition of NaCl, $MgSO_4.7H_2O$ enhance yield, while the addition of $NaNO_3$ and KH_2PO_4 depress and even inhibit yield while not affecting growth. The substitution of glucose for lactose also inhibits production.

It is suggested that penicillin production is associated with some type or phase of growth of the mould.

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Microbial Decomposition of Glutamine. By H. McIlwain

In studying metabolic activities of micro-organisms towards substances essential for their growth, it was found that β-haemolytic streptococci decomposed glutamine during glycolysis (McIlwain, 1946). The system responsible for this action has now been compared with others which react with glutamine.

The streptococcal system has required a concomitant reaction such as glycolysis, for glutamine decomposition in many types of media and over a wide range of pH values. The decomposition, and glycolysis, were slower at pH 4·5 than at pH 7, when the former proceeded at the rate of about 1–3 μmol./mg. dry wt. of organisms/hr., with the formation of nearly 1 mol. each of NH_3 and of glutamic acid/mol. of glutamine reacting. No NH_3 was formed by streptococci from some 20 compounds related to glutamine, in the

presence or absence of glucose; the organisms produced NH_3 from arginine (Hills, 1940) at a comparable rate, but independently of glycolysis. A system decomposing glutamine has not yet been separated from streptococci by autolysis or by breakdown of the cells by grinding with glass (Wiggert, Silverman, Utter & Werkman, 1940), though the latter process yielded the arginine dihydrolase in cell-free condition.

Systems resembling that of the streptococci were found in staphylococci and in *Saccharomyces cerevisiae* and *S. ellipsoideus*, but in addition these organisms were capable of a glutamine synthesis which could not be shown in the exacting streptococci.

Washed cells of strains of *Proteus morganii* liberated NH_3 from glutamine at rates (Q_{NH_3} +3 to +5 μmol./mg. dry wt./hr.) much faster than from glutamic acid, asparagine, aspartic acid, or arginine. Their reaction with glutamine was independent of glycolysis, though the organisms were capable of performing this at a high rate. Cell-free extracts containing 70% of the activity of the bacteria were easily obtained by autolysis or by grinding with glass. These were stable at 0° for several months, and when prepared by either method showed pH optima for NH_3 formation from glutamine of between 4 and 5. A precipitate formed by adding 0·38 g. $(NH_4)_2SO_4$/ml. of solution contained the glutaminase, which in solution at pH 7 or 4·5 did not dialyze through cellophane and lost its activity in $\frac{1}{2}$ hr. at 60°. This preparation at pH 4·5 did not liberate NH_3 from acetamide, asparagine, arginine, adenine, adenosine, yeast adenylic acid or glutamic acid at rates $> \frac{1}{50}$ of those at which it produced NH_3 from glutamine. It showed some glutamic acid decarboxylase activity (cf. Gale, 1940), but its rate of production of CO_2 from glutamic acid or glutamine were $< \frac{1}{10}$ those at which it produced NH_3 from glutamine.

The peculiarities of haemolytic streptococci towards glutamine in growth are thus paralleled by their metabolic activities towards glutamine.

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HCl Secretion in the Isolated Gastric Mucosa. By R. E. DAVIES

When pieces of frog gastric mucosa were incubated no production of acid was observed, although the respiration responded to histamine. This negative result could be explained by the old hypothesis of Bence Jones (1845) 'that the formation of acid sets free alkali'. His evidence was the observation of the occurrence of an 'alkaline tide' in urine. This hypothesis is supported by the work of Bulger, Allen & Harrison (1928) and Hanke, Johannesen & Hanke (1931), who showed that blood from a secreting stomach contained an increased quantity of base, and by the experiments of Delrue (1930, 1933), who first obtained HCl *in vitro* using two chambers separated by a sheet of frog gastric mucosa. No quantitative relationship between this increase of alkali and the acid secreted was established by previous workers.

The following technique was designed to combine manometric measurements with collection of secretion. The muscle layers were removed from a well-washed frog's stomach, leaving a tube of mucosa. This was tied at the oesophageal and duodenal ends and placed in a Warburg cup containing frog saline gassed with 5% CO_2 and 95% O_2 at 25°. Whilst the resultant of O_2 uptake and CO_2 output was measured manometrically for 3–8 hr., the empty bag accumulated secretion. The swollen bag of mucosa was then opened and the quantity and concentration of the acid inside were determined. The bicarbonate and lactate contents of the external fluid were also estimated.

With autumn, winter and early spring frogs spontaneous acid secretion did not occur, but after the addition of histamine to a concentration of 1 mg. % secretion occurred and was accompanied by an increased overall uptake of gas. This corresponded with an increase in the bicarbonate content of the medium which was equal to the amount of HCl secreted. This equivalence of the newly formed bicarbonate and the secreted acid verifies Bence Jones's hypothesis. Calculations based on figures given by Faurholt (1924) and Roughton (1935) show that the uncatalyzed absorption of CO_2 is too slow to deal with the alkali formed during active secretion. The necessity for the rapid neutralization of the alkali formed seems to be the physiological explanation of the presence of the large amounts of carbonic anhydrase in oxyntic cells (Davenport, 1939).

Mammalian mucosae are apparently too thick to be oxygenated effectively by saline solutions at normal temperatures, but these experiments were repeated using whole mouse stomachs at 25°.

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Relations between HCl Secretion and Electrical Phenomena in Frog Gastric Mucosa. By E. E. Crane, R. E. Davies and N. M. Longmuir

Many workers (Engelmann, 1872; Keller, 1929; Rehm, 1943) have suggested that the electric potential differences observed in glands play a part in producing secretions by electroendosmosis. In the case of gastric HCl it has been postulated that electrical energy produces acid by a reaction such as

$$Na^+ + Cl' + H_2O \xrightarrow{\text{electrical energy}} \begin{matrix} H^+ + Cl' \\ Na^+ + OH' \end{matrix}.$$

When the gastric mucosae of autumn, winter and early spring frogs were mounted in a perspex holder held between two chambers (modified from Delrue, 1930, 1933), the acid secretion induced by histamine was followed from the changes in pH of the secretory solution (see Delrue, 1930, 1933; Gray, Adkison & Zelle, 1940; Gray & Adkison, 1941) or by manometric estimation of the acid. In autumn and early spring frogs a p.d. of 30–50 mV. occurred across the membrane, the secretory side of the mucosa being negative in an external circuit.

The magnitude of the p.d. decreased with the onset of acid secretion by an amount greater for high secretion rates than for low ones. When a mucosa was connected electrically through a low resistance it supplied constant electric power (i.e. without polarization). Live mucosae obeyed Ohm's law up to approximately 1 mA./cm.^{2} applied current. Above this value the relation between p.d. and current density was again linear, but the slope (the effective resistance of 1 cm.^{2}) had a value about half that for low-current densities. This was equally true for applied currents both enhancing and opposing the natural p.d. Dead mucosae obeyed Ohm's law for all currents applied.

An important observation is that whilst enhancing currents stimulated acid production, opposing currents inhibited it. The effect increased with increasing current density and secretion could occur even when the natural p.d. was reversed in sign.

Anaerobically both the p.d. and acid secretion were abolished. HCN at 0·004 M, chloroform (saturated solution), and iodoacetate at 0·0015 M inhibited secretion and the p.d. fell to zero. Fluoride at 0·003 M had only a small effect on the p.d. and on secretion. Thiocyanate at 0·01 M raised the p.d. towards its value before secretion, and secretion was suppressed (compare Rehm, 1945; Feldberg, Keilin & Mann, 1940; Davenport, 1940).

The main result of this work is the demonstration that the rates of acid secretion can be increased or decreased by changing the p.d. across the gastric mucosa. This suggests that the electric energy of the mucosa, apparently derived from respiration, plays an important part in the mechanism of acid secretion.

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The Menstrual Cycle and Creatine-Creatinine Excretion. By G. A. MAW

Since the development of the present methods of estimation of creatine and creatinine there have been frequent references in the literature to a possible relation between the level of urinary excretion of these substances and the female sexual cycle. O. W. Smith (1942), in support of Krause & Cramer (1910, 1911), has found the onset of menstruation to be associated with either the appearance of a creatinuria or an increase in the magnitude of a creatinuria already present, and has claimed, in addition, marked cyclic variations in the excretion of creatinine.

Daily determinations of creatine and creatinine in the urines of four female subjects studied for unbroken periods of 60–70 days, with two male subjects as controls studied for somewhat shorter periods, fail to confirm Smith's results. The excretion of creatine is acknowledged to be a characteristic of the female in amounts up to 150 mg./day, but it appears to bear no obvious or consistent relation to any phase of the menstrual cycle. In the case of the four subjects studied, of the eleven periods covered, two were associated with a creatinuria, one with a premenstrual creatinuria, one with a creatine excretion lasting 18 days, and seven with complete absence of creatine excretion. This result is borne out by the data for a further ten subjects studied over menstrual periods only, creatine being present in the urine during four periods as opposed to its absence in ten.

The general character of creatinine excretion in the female appears indistinguishable from that of the male in the frequency and extent of the day to day fluctuations, in the magnitude of the standard deviations from average daily excretion values, and in the small number of statistically significant divergences from the mean. Lack of agreement with Smith's conclusions arises out of the fact that the results of this worker have been based on creatinine values obtained *periodically*, and can only be considered as approximations of the complete excretion curves which give a misleading picture of the true nature of creatinine excretion.

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Creatinine Formation in the Isolated Rabbit Kidney. By R. B. FISHER

This paper describes preliminary experiments, carried out in collaboration with Dr A. E. Wilhelmi in 1938–9.

The apparatus described by Fisher & Wilhelmi (1937) for the perfusion of the isolated rabbit heart was modified by the inclusion of a micro-version of the Dale-Schuster pump, and was used to perfuse isolated rabbit kidneys with oxygenated glucose-Ringer, in a closed circuit. Creatine and α-phosphoglycerol were added to the Ringer.

It was found that creatinine was produced during such perfusions at rates which were not less than fifty times the rate to be expected in the absence of the kidney. The actual rates of creatinine production varied widely, but the maximal rates observed, in the presence of 10–30 mg. of creatine/100 ml. of perfusate, were more than adequate to account for the daily creatinine excretion of adult rabbits.

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Observations on the Indirect Action of Ionizing Radiation on Aqueous Solutions. By W. M. DALE, J. V. DAVIES and W. J. MEREDITH

Pure enzymes have proved to be a sensitive indicator of the effect of ionizing radiations. The indirect action (dilution and protection phenomenon) of the radiation on aqueous solutions has been demonstrated using these substances (Dale, 1940, 1942; Dale, Meredith & Tweedie, 1943).

Previous experiments with crystalline carboxypeptidase have been extended to the highest concentration (14%) experimentally possible without damage to the enzyme, and show, at this concentration, an indirect effect of 75% against a direct effect of only 25%. Over a range of concentrations from 0·01 up to 14% the ionic yield is 1/6·25, which is just one-half the maximum yield by direct action assuming clusters of three ion pairs per molecule. The indirect action ionic yield for the destruction of alloxazine-adenine-dinucleotides is 1/6·9, and that of tyrosine is 1/6–1/12 (Stenström & Lohmann, 1928). These ionic yields are nearly identical, although the molecular weights of these substances vary from 35,000 (carboxypeptidase) to 181 (tyrosine).

It appears, therefore, that the ionic yield is independent of the molecular weight. The ionic yield concept directly links the number of ion pairs formed with the observed chemical change of the solute molecule. If H and OH radicals are formed from water (Weiss, 1944) as an intermediate, the figure 1/6 can be the expression of the efficiency of production of effective radicals per ion pair. By 'effective radicals' in this connexion is meant the number of radicals which have escaped recombination at their very place of origin and are now available for reaction with the solute. Hence, although the ionic yield is 1/6 the 'radical yield' may be 1/1. As an alternative to, or competing with, this process may be the capability of the solute molecules to deactivate radicals without themselves being deactivated.

The protective power of any additional solute present during irradiation is directly proportional to the molecular weight, as long as the higher members of organic compounds, which have an average uniform composition, are considered.

We have, however, found an exceptionally low protective power for the oxalate radical and for urea, a somewhat higher one for alloxan, and a very high one for the formate radical and thiourea.

The differential behaviour of these special molecules seems to lend support to the theory that free radicals are the agents of the indirect action.

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DEMONSTRATION

The Distribution of Guanin and of Fluorescent Substances in the Eyes of Fish.

By ANTOINETTE PIRIE

[*The Editors of the Biochemical Journal accept no responsibility for the Reports of the Proceedings of the Society. Abstracts of papers read are published as received from the author.*]

PROCEEDINGS OF THE BIOCHEMICAL SOCIETY

The **251st Meeting** *of the Biochemical Society was held in the Department of Biochemistry, University New Buildings, Teviot Place, Edinburgh, on Friday* 19 *July* 1946, *at* 10.30 *a.m., when the following papers were read:*

COMMUNICATIONS

The Effect of Mepacrine on the Growth and Tryptophanase Activity of *E. coli*.

By J. Dawson (introduced by F. C. Happold)

Mepacrine has been shown to be a growth inhibitor for micro-organisms. Silverman & Evans (1944) demonstrated that mepacrine inhibited *E. coli* growth, and that spermidine and spermicine removed this inhibition. Madinaveitia (1944) showed that riboflavin had an antagonistic effect on growth inhibition of *L. casei* E by various anti-materials including mepacrine. Fraser, Filadelfo & Grenan (1946) demonstrated a similar effect on *L. arabinosus* using gluathione and thioglycollic acid.

This communication demonstrates the activity of mepacrine on growth and acid production of *E. coli*, and also on the tryptophanase system.

The degree of mepacrine inhibition on growth of *E. coli*, measured by acid production, varies according to the depth of liquid in which growth occurs. In tubes 10 μg./l. mepacrine is inhibitory, whilst in flasks a minimal concentration of 30 μg./l. mepacrine is necessary. Increased concentrations of K^+ diminishes acid production in control flasks or tubes and diminishes the degree of mepacrine inhibition.

Riboflavin was not included in the basal medium. Addition of 60 μg./l. riboflavin increases acid production but does not diminish inhibition due to mepacrine in any concentration up to 60 μg./l. Further additions of riboflavin (120 μg./l.) causes no greater rise in acid production but does nullify the mepacrine inhibition.

The general conclusions drawn are that mepacrine exerts a greater inhibitory effect under moderate conditions of anaerobiosis and that riboflavin antagonizes this inhibitory effect.

The effect of mepacrine on the tryptophanase system can be described as follows:

(1) Cells grown in tryptic digest casein agar containing 0·01 % mepacrine have full tryptophanase activity.

(2) Mepacrine in culture increases the period of glucose inhibition of the tryptophanase system.

(3) Mepacrine inhibits preformed tryptophanase activity. Inhibition is complete 30 min. after incubation of washed cells with mepacrine. The initial indol production is not associated with any oxygen uptake.

(4) Washed cell suspensions (cells grown on tryptic digest casein agar) metabolizing both tryptophan and glucose show a greater initial inhibition of indole production, but at no time is the inhibition complete.

(5) Riboflavin in equimolecular proportions reduces mepacrine inhibition of tryptophanase activity.

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The Role of Folic Acid in the Growth of *Strep. lactis* R. By D. A. HALL (introduced by F. C. HAPPOLD)

Chattaway, Dolby, Happold, McMillan & Waters have shown that various growth factors for *L. casei* E, present in liver extracts, are not identical with the folic acid preparation of R. J. Williams.

Recent work (private communication) points to similar discrepancies between these fractions and the synthetic *L. casei* factor of the Lederle laboratories. The present author has also shown that the naturally occurring folic acid preparations are probably complex in nature and different from this synthetic material. Studies of the carbohydrate metabolism of *Strep. lactis* R have produced further evidence for a belief in the complexity of folic acid from natural sources. Such samples of folic acid catalyze the utilization of glucose with the production of acid by *Strep. lactis* over a large range of concentrations, only the rate and not the ultimate acid production being affected by changes in concentration. Growth also occurs in the absence of folic acid, but acid production is slight for the first 7 days, after which time it proceeds at the same rate as with the lower concentrations of folic acid. Among possible explanations of this, the concept of at least two factors associated with the folic acid complex may be considered. Attempted differentiation between the initial and delayed acid production effects of these two hypothetical components has led to the following observations. The rate at which glucose is utilized by the growing organism is not identical with the rate at which acid is produced, but bears a significant relationship to the changing rate of cell multiplication. The component responsible for the later stages of acid production may, therefore, facilitate the complete breakdown of partially metabolized glucose.

Consideration of the observations of Madinaveitia and Woolley, regarding antagonism between growth factors and similar compounds with carbon in place of certain nitrogen atoms in their structures, has provided the first successful separation of the initial and delayed acid-production effects.

On the basis of the spectroscopic data of Mitchell indicating that members of the folic acid complex have xanthopterin nuclei, inhibition of *Strep. lactis* growth was attempted with quinoxaline. Cultures on the casein hydrolysate medium used in these experiments, with no folic acid, normally showing growth after 48 hr., were blank after 7 days. The addition of folic acid, however, permitted growth, but acid production was withheld for 70 hr.

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A New Method for the Identification of Fructose in the presence of certain other Sugars. By D. J. BELL (introduced by J. S. D. BACON)

The Identification of Fructose as a Constituent of the Foetal Blood of the Sheep. By J. S. D. BACON and D. J. BELL

Noël Paton, Watson & Kerr (1907) state that: 'by extracting large quantities of foetal blood [sheep] with alcohol, evaporating with charcoal, and taking up in a small quantity of water, we were able to extract a sugar which was laevorotatory and which gave Seliwanoff's reaction distinctly....In one case the sugar in the blood was as much as 0·42 %.'

In the present investigation whole foetal blood was almost completely deproteinized by alcohol-chloroform treatment (the method used by Keilin & Mann (1940) in the preparation of carbonic anhydrase), and the water-clear extract evaporated to dryness *in vacuo*. The losses of fructose in this procedure were from 5 to 10 % of that initially present in the blood. From this residue were prepared: (1) under the conditions used by Neuberg (1902), the methylphenylosazone characteristic of fructose; and (2), in the manner described by Bell (1946), 2:3, 4:5-diacetone fructose. The yields corresponded with those expected from the amounts of fructose estimated by a colorimetric method (Mr S. W. Cole, private communication).

Measurements of the total and fermentable reducing substances (by ferricyanide-cerium), of fructose, and of glucose showed that the fermentable reducing substances in the residue corresponded with the sum of the fructose and glucose. By extracting the residue with absolute alcohol, with or without the presence of barium acetate, and evaporating to dryness, a fraction could be obtained containing most of the fructose (representing an over-all recovery of *c.* 80 % from the original blood), but considerably less of the glucose.

Examination of the optical rotation of the blood concentrate, or its alcohol-soluble fraction, showed that they were more laevorotatory than could be accounted for by their *fructose* contents, and that yeast fermentation did not remove the whole of the laevorotatory material. The change in rotation accompanying fermentation was in fairly good agreement with that calculated from the amounts of fructose and glucose disappearing.

It is therefore highly probable:

(1) that the substance in the foetal blood of the sheep giving the Seliwanoff reaction is all fructose;

(2) that the difference between the amounts of fermentable reducing substances and of fructose is accounted for by glucose;

(3) that both sugars are present mainly in the free form;

(4) that the laevorotatory power of blood extracts (cf. Orr, 1923) cannot by itself be taken as evidence for the presence of fructose.

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The Effect of Diazoaminobenzene on Vitamin A Reserves in the Rat. By A. H. M. Kirby

'Gramicidin S': the Sequence of the Amino-acid Residues. By R. Consden, A. H. Gordon, A. J. P. Martin and R. L. M. Synge

Synge (1945) showed that 'gramicidin S' consists of equimolar proportions of *l*-leucine, *l*-ornithine, *d*-phenylalanine, *l*-proline and *l*-valine; no α-amino or carboxyl groups are present. The δ-amino group of the ornithine residue is free (Sanger, 1946).

We have studied partial hydrolysates of gramicidin S (in 1:1 v/v 10 N-HCl: acetic acid at 37°) by two different approaches.

(1) The partial hydrolysate was fractionated on two-dimensional paper chromatograms (Consden, Gordon & Martin, 1944). Of duplicate chromatograms, one was coloured with

ninhydrin. The various peptides thus indicated were washed from corresponding regions cut out of the other chromatogram. The various peptides were: (*a*) completely hydrolyzed with acid; resulting amino-acids were identified by paper chromatography; (*b*) deaminated by treatment with nitrous fumes in concentrated HCl; after removal of volatile material, the product was hydrolyzed and resulting amino-acids identified.

The following dipeptides were isolated and identified in this way and then synthesized. The synthetic products agreed with the natural in their behaviour on paper chromatograms: α-*l*-valyl-*l*-ornithine, *l*-ornithyl-*l*-leucine, *l*-leucyl-*d*-phenylalanine, *d*-phenylalanyl-*l*-proline.

Two other peptide spots yielded three amino-acids each; hydrolysis products (before and after deamination) were consistent with these being α-valylornithylleucine and phenylalanylprolylvaline.

(2) The partial hydrolysate on ionophoresis (Consden *et al.* 1946) yielded two basic fractions; the faster was ornithine, the slower ornithine peptides. This last was (*a*) hydrolyzed and analyzed for component amino-acids by the method of Gordon, Martin & Synge (1943); (*b*) converted to *N*-2:4-dinitrophenyl-(DNP-)-peptides, which were separated from unsubstituted material (Sanger, 1945). After acid hydrolysis of these, the DNP-amino-acids resulting were identified by Sanger's methods, and the free amino-acids by paper chromatography.

The amino-acids of the fraction were (in decreasing molecular proportion) ornithine, valine, leucine and proline. No phenylalanine was detected. In the hydrolysate of the DNP-peptides, δ-DNP-ornithine, αδ-di(DNP)-ornithine, DNP-valine, leucine and valine were detected. Control experiments with DNP-amino-acids showed that no free amino-acids were produced under the hydrolysis conditions, and that DNP-proline was destroyed. The fraction probably consisted of α-valylornithine, ornithylleucine, α-valylornithylleucine and prolyl-α-valylornithine.

The only structure consistent with these findings is the sequence:

-α-*l*-valyl-*l*-ornithyl-*l*-leucyl-*d*-phenylalanyl-*l*-prolyl-,

occurring once or repeatedly in an endless peptide chain (cyclopeptide).

Dr D. Crowfoot and Mr G. M. J. Schmidt have examined a series of derivatives of gramicidin S by X-ray crystallographic methods. Their results are only compatible with the presence in the crystals studied either of a simple pentapeptide molecule or of a decapeptide which has crystallographic two-fold symmetry. This two-fold symmetry could arise as a result of the repetition of the peptide sequence in a cyclic decapeptide.

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Partition Chromatography on Paper as applied to the Investigation of Amino-acids and Peptides in Normal and Pathological Urines. By C. E. DENT

The applicability and potential value of one- and two-dimensional paper chromatograms (Consden, Gordon & Martin, 1944) in the study of amino-acid problems has already been indicated (Dent, 1946). The amino-acids in normal urine as revealed by this technique chiefly comprise glycine and alanine with occasional heavy traces of valine and leucine (and/or isoleucine). Pathological excess has been noted in the fatal coma of a case of

subacute hepatic necrosis, Fanconi syndrome (three cases), and in some cases of late pregnancy. It is nearly always present in acute fatal hepatic necrosis in rats whether produced by dietary means or by CCl_4. In all cases serine, threonine and 'under-alanine' are excreted although only traces have been detected in normal urines. Other amino-acids occur more variably. One case of Fanconi syndrome appeared to lack 'under-alanine' in the urine, but this is believed to have been due to decomposition. A rough quantitative estimation, by matching the strength of the ninhydrin reaction, of the 24 hr. excretion of amino-acids in the severest case of Fanconi syndrome (for description of the syndrome see McCune *et al.* 1943) gave: glycine 0·8 g., alanine 1·0 g., serine 0·25 g., threonine 0·5 g., valine 0·5 g., leucine (and/or isoleucine) 0·4 g., tyrosine 0·5 g. In addition, very variable daily excretions of aspartic and glutamic acids, arginine, hydroxyproline and phenylalanine were seen. Traces of citrulline and α-aminobutyric acid were constantly present with one or two others not yet identified.

The 'under-alanine' gave a typical ninhydrin reaction and was destroyed by hydrolysis. It was isolated by cutting out the pieces of paper containing it from sixteen two-dimensional chromatograms. Crystals were obtained in a yield of about 0·2 mg. from an original volume of 0·4 ml. of urine. Hydrolysis produced serine and glycine in the rough molecular proportions of 1:2. Destruction or blocking of the end group by HNO_2, dinitrofluorobenzene (Sanger, 1945) and HIO_4 followed by hydrolysis, left only glycine. The substance is therefore seryl-$(glycine)_n$. Pure synthetic serylglycine (from Dr J. S. Fruton) did not match the behaviour on the chromatogram. It is therefore provisionally identified as serylglycylglycine.

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The Mode of Formation of Indole from Tryptophan. By E. A. Dawes, J. Dawson and F. C. Happold

In experiments in which the overall breakdown of tryptophanase-tryptophan indole has been retarded by the action of mepacrine, the production of alanine concurrent with the liberation of indole has been demonstrated. This finding re-emphasizes the significance of the original theory of Baker & Happold (1940) which involved the preliminary fission of the alanine side chain as a whole, and its subsequent oxidative breakdown.

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The Synthesis of Nucleoprotein by Fibroblasts *in vitro*. By J. N. Davidson and C. Waymouth

When fresh explants of the chick embryo heart are grown in roller tubes by the technique of Willmer (1942), the addition of embryo extract is followed by a rise in nucleoprotein phosphorus (NPP) (Davidson & Waymouth, 1943, 1945). By suitably modifying the analytical procedure of Schmidt & Tannhauser (1945) it is possible to estimate both ribonucleic acid phosphorus (RNAP) and deoxyribonucleic acid phosphorus (DNAP) in such cultures, and the ratio of RNAP to DNAP has been found to be of the order of 4:1 or

5:1. The addition of embryo extract to cultures which have been maintained for 24 hr. in Tyrode solution alone, is, in general, followed by an immediate steady rise in RNAP, while the DNAP does not begin to rise until several hours have elapsed.

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The Action of Mustard Gas on Proteins (studied with the aid of Radioactive S). By T. E. Banks, J. C. Boursnell, G. E. Francis, F. L. Hopwood and A. Wormall

Mustard gas ($\beta\beta'$-dichlorodiethyl sulphide) reacts under physiological conditions (pH 7·5–8·0 and at 37°) with many widely differing proteins, to form compounds from which the mustard gas (M.G.) cannot be removed by long extraction with ethanol, acetone, etc. The conditions governing this reaction and the amount of M.G. combining with several proteins have been studied with the aid of M.G. containing radioactive sulphur (S^{35}).

The observations that M.G. readily reacts with serum proteins (Berenblum & Wormall, 1939), nucleoprotein (Berenblum, 1940), kerateine (Peters & Wakelin, 1941) and collagen (Pirie, 1941) have been fully confirmed.

The maximum amount of M.G. which combines with human serum proteins is about 23 mmol./16 g. of protein N, equivalent to 23 mol. of M.G./mol. of protein, assuming a mean mol. wt. of 100,000 for the serum proteins. This amount is sufficient to account for the new immunological specificity of M.G.-treated proteins. In experiments with nucleoprotein and serum albumin the S^{35} contents of the M.G.-treated proteins corresponded to 36 and 34 mmol. of M.G./16 g. of protein N respectively, but there is no reason to believe that even these values represent the maximum combining capacities of these proteins for M.G. M.G. reacts more readily with the serum proteins than it does with gelatin under comparable conditions.

The action of M.G. on certain proteins leads to a reduction of the iodine-titratable SH of the protein, but this accounts for only a small part of the reaction. Other experiments have indicated that there is no extensive reaction between M.G. and the free amino-groups of the proteins.

M.G. which has been hydrolyzed at pH 7·5 until all vesicant power has been destroyed still retains some ability to combine with proteins, but the reactivity of hydrolyzed M.G. in this respect is much smaller than that of the unhydrolyzed M.G. M.G.-sulphoxide does not combine with proteins, but there is rapid combination between mustard gas-sulphone and proteins, principally as a result of a reaction with the free amino-groups of the protein.

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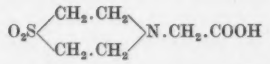
The Immunological Properties of Proteins treated with Mustard Gas and some Related Compounds. By J. C. Boursnell, G. E. Francis and A. Wormall

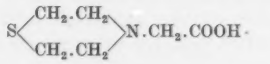
The study of the immunological properties of proteins treated with mustard gas and mustard gas-sulphone (Berenblum & Wormall, 1939) has been continued and extended. In particular, serological inhibition tests have been carried out with M.G. and M.G.-

sulphone amino-acid compounds, in order to obtain information about the immunologically dominant groups in the M.G. and M.G.-sulphone protein antigens.

Complete inhibition of the precipitin reaction between the M.G.-sulphone proteins and antisera to sulphone-proteins was effected by 'sulphone-glycine' (I) or by 'sulphone-alanine', and there was partial inhibition with a mustard gas-glycine compound (II):



I



II

This suggests that the action of M.G.-sulphone on proteins is largely concerned with the free amino-groups of the protein, as follows:

$$R.NH_2 + O_2S(CH_2.CH_2Cl)_2 \rightarrow R.N(CH_2.CH_2)_2SO_2 + 2HCl$$

The precipitin reaction between M.G.-proteins and their antisera is only slightly inhibited by (II), and in this and other respects there is a marked difference between the action of M.G. and that of M.G.-sulphone on proteins.

Divinyl sulphone, $O_2S(CH:CH_2)_2$, reacts more rapidly than does M.G.-sulphone on the serum and other proteins to give products which react, chemically and immunologically, exactly like the M.G.-sulphone proteins. Divinyl sulphide, $S(CH:CH_2)_2$, does not appear to react with the serum proteins.

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The Fate of Injected Mustard Gas (containing Radioactive S) in the Animal Body.

By J. C. Boursnell, J. A. Cohen, M. Dixon, G. E. Francis, G. D. Greville, D. M. Needham and A. Wormall

Experiments have been carried out to determine the fate of mustard gas injected intravenously into rabbits, in order to study the distribution of the substance or its products of metabolism in the tissues, and its excretion from the animal body. It was also hoped that some correlation might be found between the distribution of the injected material and the systemic damage caused by this substance. In these experiments mustard gas containing radioactive sulphur (M.G.*) has been used, and determinations have been made of the radioactive sulphur content (S*) of the blood, tissues, urine and bile of rabbits at intervals after the injection of 5 mg. of M.G.*/kg. of body wt.

The injected material rapidly diffused from the blood stream, and appreciable amounts of S*-containing compounds appeared in the urine and bile less than 20 min. after the injection.

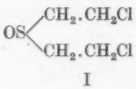
There was widespread distribution of S* in the tissues of the injected rabbits. Kidney, lung and liver gave the highest values of all the tissues examined, and it is suggested that the high values for these three organs are due to their excretory functions. The greater part, and sometimes all, of the tissue S* in these injected rabbits was not extractable by ethanol, acetone and ether, and it is believed that at least part of this 'fixed' S* was present in protein complexes.

Appreciable amounts of S* were excreted in the urine and bile of the injected rabbits, and it is suggested that the latter mode of excretion might account for at least part of the intestinal damage occurring in M.G. poisoning.

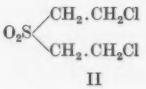
No evidence has been obtained of preferential retention of the injected M.G., or its S*-containing derivatives, in the fatty tissues of the body, or in any organ, with the exception of kidney, lung and liver.

The Fate of Mustard Gas-Sulphone and Mustard Gas-Sulphoxide (containing Radioactive S) in the Animal Body. By J. C. BOURSNELL, G. E. FRANCIS and A. WORMALL

In view of the possibility that mustard gas (M.G.) might undergo oxidation in the animal body, injection experiments similar to those described in the preceding paper have been carried out with two oxidation products of M.G., viz.: M.G.-sulphoxide (I) and M.G.-sulphone (II). As in the experiments with M.G., the radioactive S^{35} was used as a tracer element, and the sulphoxide* and sulphone* were prepared from S^{35}-containing M.G. (M.G.*):



I



II

The injected sulphoxide* or sulphone* was fairly rapidly distributed throughout the body, and appreciable amounts of radioactive sulphur (S*) were found in all the tissues examined 1 hr. after the injections. The injected rabbits also excreted appreciable quantities of S* in the urine and/or bile.

A comparison of the results of the experiments with M.G.*, M.G.-sulphoxide* and M.G.-sulphone* suggests that the three substances are similarly distributed throughout the body after injection, and that they all react with the tissues to form acetone- and ether-insoluble S*-containing compounds ('fixed' S*). This reaction may be partly or wholly concerned with the tissue proteins in the case of M.G.* and M.G.-sulphone*, but probably not with M.G.-sulphoxide*, for this compound, unlike M.G.* and its sulphone, does not react or combine with proteins, e.g. of human serum, in *in vitro* experiments carried out under physiological conditions of pH and temperature.

Some Aspects of the Metabolism of Arginine in Malignant Tissue. By S. J. BACH and I. LASNITZKI

The abundance of arginine in the proteins of tissues connected with reproductive functions is a striking phenomenon. The protamines in fish sperm, for example, contain up to 90 % arginine, and it has even been suggested that the arginine content of a cell may serve as a measure for nuclear division and particularly so in fast-growing tissue such as malignant tumours. This problem, which has caused some controversy in the past, was reinvestigated in *in vitro* experiments using the technique of tissue culture.

Mouse adenocarcinoma was grown in media containing different concentrations of arginine for various experimental periods, after which the cultures were fixed and a differential cell count was made in order to compare the mitotic index of cultures, grown with and without arginine. It was observed that the mitotic index was increased by 100–200 % over the control cultures without arginine on the 1st and 3rd day of incubation. The arginine effect was absent when, in place of malignant tissue, normal lung cultures from mouse embryos were grown in parallel experiments.

The question whether arginase as a participant in one of the metabolic reactions of arginine caused a stimulation of mitosis was investigated in two ways: First, it was shown that an inhibition of arginase by ornithine (Bach, Crook & Williamson, 1944) increased the mitotic index of malignant tissue while it had no effect on normal lung tissue. This observation can be interpreted as evidence for a growth-retarding action of arginase in malignant tissue by means of which the tissue may be deprived of a specific growth factor, and by the inhibition of which action, an arginine-saving effect may be exerted.

This hypothesis was put to the test by a further series of 20 experiments in which the arginase contents of carcinomatous tissues of various growth rates were determined. The results show clearly that slowly growing tumours contain twice as much arginase as fast growing tumours, which again suggests that arginase, far from stimulating malignant growth as has generally been assumed in the past (Edlbacher & Merz, 1934), actually produces a retardation.

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Arginase and Creatinuria in Malignant Tissue. By S. J. Bach

The urinary nitrogen distribution of Jensen sarcoma rats and normal rats was studied and an 80 % increase in the creatine-N fraction of the tumour rats over that of the control rats was observed. The total nitrogen excretion and the creatinine-N as percentage of the total-N was the same in the normal and tumour rats while the percentage urea-N was slightly decreased in the tumour rats.

In the light of the previous communication (see above) these findings suggest a connexion between malignant growth and the metabolism of arginine to creatine as distinct from the action of arginase on arginine which exerts a retarding effect on tumour growth. This suggests the possibility of an antagonism with respect to malignant growth, between arginase action and creatine synthesis from arginine. It was the object of a few preliminary experiments to test this possibility by injecting a pure arginase solution intravenously into sarcoma rats in the hope of observing changes in the urinary nitrogen distribution as compared with sarcoma rats without injections.

For this purpose an attempt was made to purify arginase from liver extracts and eventually an enzyme solution of high $Q_{\text{urea-CO}_2}$ purity (Q urea-CO^2 = 40,000 at 37°) was prepared from which enzyme crystals could be obtained.

In the single series of experiments performed so far, a powerful enzyme preparation was injected into one set of tumour rats causing a redistribution of urinary nitrogen on the days following the injection: the urinary creatine-N fraction of the injected animals was considerably lowered as compared with the uninjected animals while the urea-N fraction was slightly increased. In the 'recovery period' following the days of injection the conditions were reversed: the creatine-N fraction of the injected tumour rats exceeded that of the control tumour rats and the urea-N fraction was slightly lower.

Owing to the exceptionally slow growths of the particular tumours of both the enzyme treated and control rats, no conclusion can be drawn with regard to the effect of the arginase injections on the growth rate of the tumours, though a slight indication of a transitory slowing effect of the injected enzyme on the tumour growth could be observed. However, the fact that the enzyme appears to have caused a divergence of the nitrogen metabolism in the sarcoma rats is noteworthy though more confirmatory experiments are needed.

Synthetic Media for Penicillin Production. By R. P. Cook and Margaret B. Brown

From the results obtained by the use of fractions prepared from either aqueous extracts of ground dried peas or of green peas (Cook & Brown, 1946) we have attempted to formulate a synthetic medium which would give high yields of penicillin. Although our main experiments have been carried out using *Penicillium notatum* NRRL, 1249, B 21 as a surface culture, we have also used *P. notatum* NRRL, 2000 (× 1612) in submerged aerated

culture. The basal medium contains (as g./100 ml.) $NaNO_3$ 0·3, KH_2PO_4 0·05, $MgSO_4.7H_2O$ 0·025, NaCl 1·0, lactose 3·0, to which the various substances under test were added. The chemical compounds were added in such quantities that a total dry matter concentration was obtained in the medium which would correspond to the dry matter present when using our most active fraction. Nitrogen-containing compounds when used alone were added so as to give a value of 140 mg. N/100 ml. of medium.

The addition of individual amino-acids alone has only a slight stimulating action, but with the simultaneous addition of glucose (1·5 %) leucine and tyrosine give high yields of penicillin. There was no correlation between penicillin formation and mycelial growth.

We have found that gelatin in amount sufficient to give the equivalent of 140 mg. N/100 ml. of medium provides a good source of mixed nitrogen-containing compounds. There was only slight mycelial growth on the medium containing gelatin alone but on the addition of various carbohydrates or compounds related to them, good yields of penicillin were obtained. The addition of sugar alcohols such as glycerol gave yields of 160 units/ml.; monosaccharides in general have only a slight stimulating effect; of the disaccharides and trisaccharides tested raffinose (at a concentration of 1·5 %) gave the highest yields.

The yields on the gelatin raffinose medium may be enhanced by increasing the amounts of NaCl and lactose in the medium. Thus 2 % NaCl and 4 % lactose in this medium gave 300 units/ml. in 8 days.

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The Relationship of Folliculosis to Vitamin A and Ascorbic Acid. By D. G. McIntosh, T. Moore, D. M. Keay and R. P. Cook

During the course of a dietary survey in the period January–March 1944, of a group of 45 boys, aged 14–15, a high incidence (29 %) of the skin condition known as folliculosis was observed (Cook, Davidson, Keay & McIntosh, 1944). This condition is said to be an earlier stage of follicular keratosis which is regarded by many writers as a manifestation of avitamosis A (Loewenthal, 1933; Nicholls, 1933; Goodwin, 1934; Pemberton, 1940; Sefton, 1946), although an identical condition is claimed to occur as an early sign of avitaminosis C (Wiltshire, 1919; Scheer & Keil, 1934; Crandon, Lund & Dill, 1940).

If these two vitamins are to be implicated chemical estimations of their content in the plasma might be expected to show low values.

Values of various blood constituents in groups of pubescent boys; and children attending a day nursery

Period	No. examined	Showing folliculosis			Not showing folliculosis		
		No. in group	Range	Mean	No. in group	Range	Mean
		Plasma vitamin A, i.u./100 ml.					
Jan.–Mar. 1944	43	13	54–147	81·85	30	29–139	78·87
Dec. 1945	60	23	39–135	86·74	37	51–197	89·57
April 1946	15	2	98–136	117·0	13	40–115	71·30
		Plasma carotenoids, i.u./100 ml.					
Jan.–Mar. 1944	43	13	44–135	79·85	30	24–178	84·33
Dec. 1945	60	23	66–228	106·78	37	53–170	98·81
April 1946	15	2	157–168	162·50	13	62–234	105·38
		Plasma ascorbic acid, mg./100 ml.					
Jan.–Mar. 1944	45	13	0·05–0·30	0·18	32	'Nil'–0·30	0·12

An additional group of 60 boys (mean age, 15; incidence of folliculosis, 38%) and a group of 15 day-nursery children (ages 2–4; incidence of folliculosis, 13%) were examined during December 1945 and April 1946 respectively. The results obtained in the three groups are shown in the table.

Therapeutic tests involving the administration of ascorbic acid and of vitamin A have also been carried out, and showed that the vitamins had no effect on the skin lesions.

Our findings endorse the view of Stannus (1945) that the follicular lesions commonly encountered in this country are those of keratosis pilaris which has no direct association with avitaminosis A or C. He suggests that it is a reaction in a predisposed skin to a disturbance in its normal metabolism.

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Studies on Histaminase. By R. Kapeller-Adler

Various workers are agreed that histaminase is a flavoprotein. The prosthetic group appears to be flavin adeninedinucleotide but the reversible resolution of the enzyme is very difficult since the protein is easily denatured. Zeller (1938) claimed that histaminase was not specific for histamine, but also oxidized diamines such as cadaverine, putrescine and agmatine. He therefore proposed that the enzyme should be called diaminoxidase. It has been found that these various substrates did not always give identical results when tested with serum from cases of pre-eclamptic toxaemia or with extracts of placenta (Kapeller-Adler, 1944). Attempts have therefore been made to separate two enzymes in extracts of hog's kidneys. Many of these experiments were not inconsistent with Zeller's theory, but when the purified enzyme was dialyzed, its action on histamine was decreased while its action on cadaverine, putrescine and agmatine was increased. An acetone extract of the purified enzyme was dried *in vacuo* and taken up in phosphate buffer. This extract reversed both the effects of dialysis.

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Sugars in the Foetal and Maternal Bloods of Sheep. By S. W. Cole and M. W. S. Hitchcock

The presence of fructose in foetal blood has been described by authors as far back as Claude Bernard (see Needham, *Biochemistry and Morphogenesis*, p. 616), but the quantity present has not been determined systematically throughout the gestation period.

Estimations have therefore been made by Cole's modification of Roe's method (Roe, 1934) of the ketose (now proved to be fructose by Bell and Bacon) in the blood of the pregnant ewe and foetus respectively, and also of the total blood sugar. The difference is assumed to be glucose.

Foetal age (days) ...	72	76	78	102	102	110	121	122	135	136	142	144
Fructose mg. %:												
Umbilical artery	—	100	—	106	88	66	134	50	79	54	51	54
Umbilical vein	143	103	108	108	84	69	146	47	80	54	93	51
Uterine artery	2	1	1	1	1	1	1	1	1	1	1	1
Uterine vein	3	1	—	1	1	2	2	1	1	1	1	1
Amniotic fluid	—	—	—	188	124	130	363	90	132	70	81	103
Total sugar mg. %:												
Umbilical artery	—	143	—	131	124	90	198	69	130	82	67	70
Umbilical vein	181	149	156	145	121	94	158	84	130	86	105	75
Uterine artery	81	110	59	74	71	59	92	51	110	57	72	61
Uterine vein	58	88	—	59	67	35	76	42	82	58	40	42
Amniotic fluid	—	—	—	308	237	145	420	226	—	172	110	303
Jugular vein before operation (maternal)	—	—	—	—	46	35	42	24	—	14	36	29

The following determinations were made on the blood from the jugular vein of lambs:

	Lamb 1	Lamb 2		
Age ...	3 days	1 hr.	24 hr.	6 days
Fructose mg. %	2	27	3	2
Total sugar mg. %	82	73	94	58

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The Diagnostic Significance of Capillary Strength Measurements. By S. Lazarus, H. N. Munro and G. H. Bell

Positive and negative pressure tests for capillary strength were applied to four different groups: (1) students (mean age 20 years), (2) hospital visitors (mean age 65 years), (3) hospital cases without scurvy (mean age 74 years), and (4) cases of frank scurvy (mean age 62 years). Between these groups exist differences in age, social status and vitamin C intake.

The results with the positive pressure technique are little affected by age, and in groups 3 and 4 the mean values are significantly different. This indicates that a special degree of capillary weakness can be demonstrated in scurvy with the positive pressure test. However, wide variations in the results obtained in individual cases make this test of little value as a diagnostic procedure.

With the negative pressure test, the results are greatly altered by age and no difference was found between groups 3 and 4. The test is therefore of no value in the diagnosis of frank scurvy.

Idiopathic Hypoparathyroidism treated with Calciferol and Calcium. By J. C. Eaton

A man of 27 years of age who had had tetanic attacks for 15 years was found to have low serum calcium and high inorganic phosphate. Treatment with calciferol (90,000–200,000 i.u. daily) and calcium by mouth resulted in complete freedom from tetany and raised the

serum calcium to normal levels. Sixty-four days after this treatment commenced, however, it was found on radiological examination that calcification of peripheral arteries had occurred. Despite cessation of calciferol administration this persisted. Since tetany recurred after stopping calciferol it was decided to give a modified dose with calcium. Although the dose during 12 months did not exceed 30,000 i.u. daily, calcification of the arteries has tended to increase slightly, but the patient has been almost free from tetany. Plasma inorganic phosphorus has remained high even when the treatment resulted in a normal serum calcium.

The possibility that this patient is a case of pseudo-hypoparathyroidism described by Albright, Burnett, Smith & Parson (1942) cannot be entirely excluded.

REFERENCE

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Imbalance of Vitamin B Factors. Conditioned pyridoxine deficiency and its effect on thymus weight of weanling rats. By M. B. RICHARDS (introduced by D. P. CUTHBERTSON)

The occurrence of fits in the litters of rats on a synthetic diet with high B_1 content has previously been reported (Richards, 1945). The conditions of the experiments and the fact that no fits were observed in the litters of does which received 40γ pyridoxine daily from parturition appeared to indicate that a pyridoxine deficiency had been induced by the excessive intake of vitamin B_1. At all levels of B_1 the addition of chalk to the diet appeared to increase the B_6 deficiency.

In subsequent experiments where the imbalance of the diet was accentuated by a further increase in the intakes of vitamin B_1 and chalk, there was no marked effect at first on the growth or clinical condition of young rats, but prolongation of the tests for several months indicated the presence of irreparable damage in some animals. Fits occurred in adult rats, both male and female, which could not be cured by subsequent administration of pyridoxine. The number of still-births and difficult parturitions increased greatly, and some females proved quite incapable of rearing their young, even when given pyridoxine. With females which had suffered less severely it was possible, by administering and withholding pyridoxine alternately, to get apparently normal litters alternating with litters which showed the characteristic fits of pyridoxine deficiency.

Very marked differences were found in the thymus weights of the weanling rats from these alternate litters. Both absolutely, and relatively to body weight, the thymus weights of weanlings suffering from the conditioned pyridoxine deficiency were much below those attained when the mother had been given pyridoxine during lactation. Thus for one doe with typical behaviour the average thymus weights in g./100 g. body wt. for successive litters without and with pyridoxine were 0·234, *0·396*, 0·182 and *0·356*, the values for the pyridoxine litters being in italics. For a number of stock litters examined the average value found was 0·350 g. Other workers have reported partial or complete atrophy of the thymus in rats fed after weaning on diets lacking in pyridoxine. The present results show that such atrophy may occur even before weaning, and that an adequate supply of pyridoxine during the suckling period is essential to ensure normal development of the thymus at this stage. These and other experiments on vitamin B imbalance give indications of other conditioned deficiencies, and the possible effect of such deficiencies on thymus weight is also under investigation.

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DEMONSTRATION

The Rapid Approximate Determination of the Solubilities of Solids in Hot and Cold Solvents. By W. KLYNE and C. BELL

Few papers on biochemistry or organic chemistry give even roughly quantitative data on the solubilities of new compounds described. A method for obtaining such data at various temperatures with a minimum expenditure of time and material will be demonstrated.

The apparatus used consists of a small flask and an adapter carrying a small burette and a reflux condenser. The substance is placed in the flask; the solvent is then run in from the burette while the flask is heated on the water bath or sand bath until the solid just dissolves in the boiling solvent. This gives the solubility at the boiling-point. The solution is then cooled to room temperature or to 0°, and filtered from the solid which has separated. A measured volume of the filtrate is evaporated to dryness, giving the solubility in the cold.

Results for known compounds obtained by this method show fair agreement with those given in the literature. A pair of determinations by this method can be carried out in two periods of about 30 min. working time each, and the material can easily be recovered for further work.

[*The Editors of the Biochemical Journal accept no responsibility for the Reports of the Proceedings of the Society. Abstracts of papers read are published as received from the author.*]

PROCEEDINGS OF THE BIOCHEMICAL SOCIETY

The **252nd Meeting** *of the Biochemical Society was held in the Department of Biochemistry, The University, Liverpool, on Friday, 27 September* 1946, *at* 11 *a.m., when the following papers were read:*

COMMUNICATIONS

A Further Sulphuric Acid Ester from the Urine of Pregnant Mares. By W. KLYNE

Klyne & Marrian (1945) described the isolation from mare's pregnancy urine of the sulphuric acid ester of a steroid of the probable formula $C_{21}H_{32}O_2$, now called 'Compound *Z*'. Comparison of *Z* acetate with the acetate of Δ^{16}-*allo*pregnene-3(β)-ol-20-one indicates that the two substances are possibly but not certainly identical.

A further sulphuric acid ester has now been obtained from mare's pregnancy urine by methods similar to those previously used. The *p*-toluidine salt forms fine white needles, m.p. 181°–183° (dec., corr.), $[\alpha]_D^{20°} = +16° \pm 4°$ ($c = 3$ in ethyl alcohol), and has the probable formula $(C_{21}H_{35}O.SO_4)^- (CH_3C_6H_4.NH_3)^+$.

This salt on hydrolysis yields a compound of the probable formula $C_{21}H_{36}O_2$ (Compound *Y*) which forms needles from aqueous ethyl alcohol, m.p. 211°–213° (corr.), $[\alpha]_D^{20°} = +2° \pm 8°$ ($c = 0{\cdot}87$ in ethyl alcohol). It is precipitated by digitonin, and is therefore probably a 3(β)-hydroxy steroid. Compound *Y* forms a diacetate (see below) and is completely resistant to catalytic hydrogenation. It is oxidized by chromic acid to a compound $C_{21}H_{32}O_2$ (m.p. 171°–173°, corr.) which forms a *mono*-semicarbazone (m.p. 217°–221°, dec. corr.).

The diacetate ($C_{25}H_{40}O_4$) forms plates from aqueous methyl alcohol, m.p. 159·5°–160·5° (corr.), $[\alpha]_D^{19°} = -32° \pm 4°$ ($c = 3$ in chloroform).

All the above data suggest that Compound *Y* is a saturated diol isomeric with pregnanediol. The properties of *Y* and its derivatives do not agree with those of any known pregnanediol or *allo*pregnanediol. They do, however, agree with those of the so-called 'uranediol' of Marker, Rohrmann & Wittle (1938).

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The Relationship of Histidine to the Folic Acid Group of Growth Factors. By D. A. HALL

In the course of experiments regarding the mode of action of folic acid, attempts have been made to replace the factor by simpler compounds. Stokes (1944) and others have reported the replacement of folic acid by thymine, while Chattaway (1944) has shown that orotic acid is active for *L. casei*. It has also been demonstrated that both purine and pyrimidine bases are necessary for the adequate growth of *Strep. lactis* R. For some considerable time there has been a certain amount of evidence that histidine acts as a precursor for these bases in mammalian metabolism, and experiments have been carried out to determine whether there is a similar connexion at the bacterial level.

It has been shown that when added to casein hydrolysate culture media in large quantities before sterilization, histidine has the power of replacing folic acid as a growth and acid producing factor for *Strep. lactis* R. It is, however, not identical with this factor, since its effect on the acid production of the organism depends on the relative concentration of both histidine and folic acid when added simultaneously. The increase in concentration of histidine required to induce a given change in acid production bears a

fractional power relationship to the concentration of folic acid required to produce an identical effect. This, when considered in conjunction with the fact that histidine is almost inactive when added aseptically to culture media, points to the interaction of histidine with some component of the medium during steam sterilization, to produce a compound having qualitatively similar properties to folic acid.

The effect of the simultaneous addition of histidine and folic acid is that of increasing the initial rate of acid production without affecting the ultimate amount of acid produced. The inclusion of folic acid and the synthetic *L. casei* factor produces an identical effect, which, coupled with the partial reversal of quinoxaline inhibition effected by histidine, may point to the possible production during autoclaving of a compound containing a pyrimidine ring. Theoretically the normal processes of electronic redistribution in the iminazole ring, which might be induced by interference in the electronic balance of the α-amino carboxyl doublet, could result in a process of ring expansion with the production of a 4-substituted pyrimidine. It is therefore interesting to note that orotic acid which has a similar though oxidized structure, although not active alone as a factor inducing acid production by *Strep. lactis* R, does act synergistically in this respect in the presence of folic acid during the early stages of growth.

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Further Data on the Action of Azo-compounds in Rats and Mice. By A. H. M. Kirby

Proliferative lesions could not be obtained in stock mice by feeding diazoaminobenzene (DAAB) in a balanced or in a modified diet, nor by subcutaneous injections of an arachis oil solution. However, painting with an acetone solution caused very marked hyperkeratosis, with hyperplasia of the epidermis and dermis, and ulceration. In two mice, a male dying after 346 days' painting and a female after 601 days, horny growths were induced which were found to be squamous carcinomas invading connective tissue and muscle fibres. No neoplastic lesions were seen in the livers of mice receiving DAAB by any route, and it seems that there is no *in vivo* conversion of DAAB to *p*-aminoazobenzene.

p-Aminoazobenzene has already been reported to cause liver cancers in Wistar rats given the compound orally in a diet containing boiled potatoes (Kirby, 1944). An attempt to repeat this work with rats of the same origin, but using a diet containing starch instead of potatoes, has failed to yield any proliferative lesion of the liver. However, similar rats fed with the same starch diet supplemented with *N*:*N*-dimethyl-*p*-aminoazobenzene (DAB) had an incidence of liver tumours of only 25% at 1 year; rats fed with DAB in the potato diet had an incidence of 100% at 10 months. Hence the starch diet was protective as compared with the potato diet.

Wistar rats fed with the ammonium salt of the formaldehyde-bisulphite derivative of *N*-monomethyl-*p*-aminoazobenzene developed only cholangiectasis even after 2 years' feeding. Apparently the formaldehyde-bisulphite group is not readily removed *in vivo*.

N:*N*-Diethyl-*p*-aminoazobenzene (EAB) given orally to Wistar rats up to 21 months, failed to induce any hyperplastic changes in the liver; this agrees with results of American workers.

DAB is known to be largely demethylated in the liver prior to reductive fission. EAB is probably not de-ethylated. Hence reductive fission will yield *N*:*N*-diethyl-*p*-phenylenediamine. The latter has been shown by Elson & Hoch-Ligeti (1946) to be inhibitory

towards urease. If the 'split-product' hypothesis of Kensler, Dexter & Rhoads (1942) were true, then EAB should be carcinogenic for rat liver.

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The Relationship between Blood Vitamin A Levels and Liver Stores in Rats. By J. GLOVER, T. W. GOODWIN and R. A. MORTON

Neither in humans nor in animals has any simple relationship been convincingly demonstrated between vitamin A plasma levels and liver stores. The data of Lewis, Bodansky, Falk & McGuire (1942) and Josephs (1942) imply that plasma levels are controlled by a mechanism to some extent independent of the liver reserves. Lewis *et al.* found that plasma levels of rats remained between 37 and 53 i.u./100 ml. even when there was little or no liver storage, whilst Josephs found that post-absorptive plasma levels were only slightly raised even after large doses of vitamin A. Plasma vitamin A is normally in the free (alcohol) form (Clausen, Baum, McCoord, Rydeen & Breese, 1942) whereas that stored in the liver is overwhelmingly ester, although small amounts of free vitamin A are always present (Gray, Hickman & Brown, 1940). It seemed possible that the liver level of unesterified vitamin A is the variable controlling the plasma level. This hypothesis was tested by measuring concurrently the plasma levels and free and esterified liver levels of suitably dosed rats.

Using a photoelectric spectrophotometer (Beckman) it was possible to measure accurately the vitamin A content of plasma when only small amounts were available (2–5 ml.). Separation of free and combined vitamin A was achieved chromatographically using defatted bone-meal (Mann, 1943) as adsorbent. The esterified vitamin A is eluted with 2·5% (v/v) chloroform in petrol, and the free vitamin A with pure acetone.

Table 1 is a selection from the data which lead to the conclusion that plasma vitamin A levels are proportional to the free vitamin A liver levels and not to total liver storage.

Table 1. *The relation between vitamin A plasma levels and free and total liver levels in rats*

i.u. administered	i.u./100 ml. plasma	i.u./g. liver	
		Total	Free
—	38	71	13
10,000	36	307	22
50,000	57	3,606	131
150,000	67	4,030	145
500,000	147	13,500	618
1,000,000	86	16,580	271

It is also evident that a mechanism, as yet obscure, tends to maintain the vitamin A *status quo* in the blood by attempting to keep constant the free vitamin A liver levels for the percentage of the total stores existing as free vitamin A is much less when storage is large than when it is negligible, e.g.

total stores 13,500 i.u./g. % free vitamin A 5%,
total stores 4 i.u./g. % free vitamin A 65%.

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Accurate Determination of Vitamin A. By R. A. MORTON and A. L. STUBBS

$E^{1\%}_{1\,cm.}$ 328 mμ values for vitamin A oils need correction, since even the richest commercial preparations show irrelevant absorption. Given the curve for pure vitamin A and extinctions at 313, 328 and 338·5 mμ on oils, measured by photoelectric spectrophometry, the vitamin A contribution can be determined (Morton & Stubbs, 1946).

The correction (av. 6%) for molecular distillates is smaller than that for oils (11–16%). Variations between samples, even from a single species, show that a precise figure for vitamin A potency requires a corrected E value.

Two halibut-liver oils used in cooperative assays (Hume, 1937, 1939; Irwin, 1944) have been re-examined after storage for 10 years. One had not changed detectably, and after correction of E values, the apparent conversion factor of 1570 becomes 1800. This agrees with the factor 1770 reached (Hume, 1943; Irwin, 1944) on vitamin A-naphthoate (cryst.) which apart from a trace of A_2 was pure. Recent samples of vitamin oils gave the following results:

	No. tested	$E^{1\%}_{1\,cm.}$ 328 mμ av.	$E\frac{\text{corr.}}{\text{gross}}$	Apparent conversion factors accepting 1800 as true factor av.
Commercial distillates	16	100	0·86–0·99	1700
Very rich oils	12	200	0·79–0·89	1500
Rich oils	20	100	0·80–0·90	1550
Halibut-liver oils	16	50	0·83–0·97	1590
Shark-liver oils	15	25	0·77–0·90	1510
Hake-liver oils	6	5	0·85–0·93	1610
Dogfish-liver oils	3	6	0·82–0·94	1560
Other liver oils	20	25	0·70–0·94	1540

The factor of 1600 remains appropriate enough for uncorrected E values, but 1800 should be used for corrected E values. The need for correction arises from the presence of anhydrovitamin A and sometimes oxidation products.

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Vitamin A in Whale Liver Oil. By R. A. MORTON and A. L. STUBBS

Whale-liver oils are very variable, particularly because of the presence of kitol and 'cyclized' or anhydro-vitamin A and accurate assessment of potency has been almost impossible. Correction of absorption curves obtained by photoelectric spectrophotometry reduced a gross value for $E^{1\%}_{1\,cm.}$ 328 mμ from 93·3 to 64·9, and a difference curve agreed with that of kitol. Chromatography divided the oil into an ester fraction (88·7%, $E^{1\%}_{1\,cm.}$ 56·8 gross, 34·1 corr.) and an alcohol fraction (9·6%, $E^{1\%}_{1\,cm.}$ 331 gross, 273 corr.). In general the best procedure for crude oils is to partition the unsaponifiable fraction between petroleum ether and 83% ethyl alcohol, whereupon anhydrovitamin A remains in the petrol and kitol and vitamin A pass into the aqueous alcohol. The vitamin fraction (in cyclohexane) gives accurately correctable curves. The corrected $E^{1\%}_{1\,cm.}$ 328 mμ value is multiplied by 1800 to give the potency in i.u./g. Refined whale-liver oil preparations, however, give the same result when the E 328 mμ values on simple solutions are corrected from measurements at 313, 328 and 338·5 mμ.

Ling Cod and Other Fish Liver Oils Rich in Vitamin A_2. By R. A. MORTON and A. L. STUBBS

Ling cod-liver oil is a very rich source of vitamin A (up to 10% vitamin). In the antimony trichloride colour test, maxima at 617 and 693 mμ are shown, the relative intensities being very constant at 7–8/1. If the ultra-violet absorption curve is plotted and the true E value for vitamin A obtained from readings at three wave-lengths, the whole curve for vitamin A can be drawn. If this is subtracted from the curve for the oil, the difference is the absorption of vitamin A_2 plus any other irrelevant absorption. Oils subjected to a preliminary chromatographic purification, show $A/A_2 = 7{\cdot}5:1$ by the correction procedure and by the colour test.

The desterolated unsaponifiable fraction of ling cod-liver oil contains some 10% vitamin A_2 and provides the most easily accessible source of this vitamin.

The commercial oils known as 'Red' and 'Black' cod-liver oils are also rich in A_2.

Retinene$_1$-Vitamin A Aldehyde By S. BALL, T. W. GOODWIN and R. A. MORTON

Retinene$_1$ described as the prosthetic grouping of visual purple (rhodopsin) was discovered by Wald (1935–6) but remained known only by its spectroscopic criteria ($\lambda_{max.}$ 365 mμ in petrol, 385 mμ in chloroform, 664 mμ $SbCl_3$ colour test). Morton (1944) argued that it was vitamin A aldehyde and Morton & Goodwin (1944) obtained a spectroscopically homogeneous preparation in poor yield by careful oxidation of vitamin A alcohol by permanganate. Hunter & Hawkins (1944) then described a preparation of vitamin A aldehyde, the product being likewise substantially homogeneous.

Retinene$_1$ is now made as follows: Sterol-freed non-saponifiable matter from rich fish liver oil ($E^{1\%}_{1\,cm.}$ 328 mμ preferably > 100) is dissolved in purified petroleum ether and left to stand in the cold over manganese dioxide for 3–4 days. The 328 mμ maximum due to vitamin A gradually diminishes in intensity and is replaced by a maximum at 365 mμ (petrol) or 385 mμ (chloroform). The 617 mμ band in the $SbCl_3$ colour test disappears and a 664 mμ maximum takes its place. The progress of the change is followed spectroscopically, and when the 664 mμ band reaches maximum intensity the solution is filtered and subjected to purification by chromatographic adsorption on alumina. Preparations showing:

$\lambda_{max.}$ 370 mμ	$E^{1\%}_{1\,cm.}$ 1200
$SbCl_3$ $\lambda_{max.}$ 664 mμ	$E^{1\%}_{1\,cm.}$ 3300

are regularly prepared.

Attempted crystallization has yielded an impurity $\lambda\lambda_{max.}$ 325, 280 mμ (weak absorption). The aldehyde gives a cryst. 2,4-dinitrophenylhydrazone, analyzing correctly but from its absorption spectrum it does not appear to be normal. The aldehyde gives a silver mirror with Tollens's reagent, but the reaction is complicated.

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Retinene$_2$-Vitamin A_2 Aldehyde. By R. A. MORTON, M. K. SALAH and A. L. STUBBS

Wald (1937) discovered retinene$_2$ in studying porphyropsin, the visual purple analogue, which, like vitamin A_2, occurs in the retinas of fresh-water fishes. Its existence was postulated because, instead of the 385 mμ ($CHCl_3$) maximum of retinene$_1$, $\lambda_{max.}$ occurred at 405 mμ, and the 664 mμ colour test band was displaced to 705 mμ.

Vitamin A_2 from fresh-water fish livers (e.g. pike) may be obtained relatively free from A but in small amount. Many salt-water fish-liver oils contain A_2, but the ratio A/A_2 is usually >15. Ling cod-liver oil shows A/A_2 *c.* 8:1, but some samples are very rich ($E^{1\%}_{1\,cm.}$ 328 mμ *c.* 200) in vitamin. Attempts to decrease the A/A_2 ratio by fractionation and by chromatography succeeded, but a complete separation of the two vitamins has not so far been possible. Desterolated unsaponifiable matter ($E^{1\%}_{1\,cm.}$ 328 mμ 820·4 g.) is dissolved in petrol and left to stand in the cold over MnO_2. The 617 mμ and 693 mμ maxima in the $SbCl_3$ colour test gradually give place to maxima at 664 mμ and 735–740 mμ (intensities 10:1). Chromatographic adsorption on alumina (Spence) gives some separation but a red artefact is a major complication. Weakened alumina is more useful and by combining fractions from many runs, the 735–740 mμ chromogen can be gradually accumulated and finally separated completely from the 664 mμ chromogen. The ultra-violet absorption maximum, initially at 370 mμ (retinene$_1$ predominating) gradually moves in the direction of longer wave-lengths with progressive increase in the concentration of retinene$_2$, until it reaches 385 mμ (petrol) and 405 mμ (chloroform).

The $SbCl_3$ test shows $\lambda_{max.}$ at 735–740 mμ initially for retinene$_2$, but, on standing, shifts to 700 mμ. This curious effect is most marked when retinene$_2$ has been practically freed from retinene$_1$.

Both retinene$_1$ and retinene$_2$ have been prepared by a gentle method at a solid surface, the oxidation affecting only the primary alcohol groups of vitamins A and A_2. Both form (spectroscopically abnormal) 2-4 dinitrophenylhydrazones. The vitamin (A or A_2) *alcohol* $\rightleftharpoons$ retinene (1 or 2) *aldehyde* oxidation-reduction systems in scotopic vision have thus been simulated *in vitro*.

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The Transport of Absorbed Carotene in Herbivora. By T. W. Goodwin, A. D. Dewar and R. A. Gregory

The ingestion of active carotenoids by animals leads to vitamin A storage in the liver, the pro-vitamin being converted to the vitamin in that organ. The cow appears to be the only herbivorous animal which has carotene circulating in the blood plasma. These carotenoids are not functional but merely in transport to the liver, and the plasma levels reflect the intake of carotenoids in the foodstuffs. Goats and sheep, on the other hand, never have carotenoids present in their blood plasma (or if so, only in minute quantities, e.g. 1–2 μg./100 ml.). This suggests that the conditions of absorption and/or mode of transport of carotenoids in these animals is different from that in cows, with the possibility that in the latter it is via the lymphatics whilst in the former it is via the portal system.

Experiments are in progress to ascertain the route of absorption of carotenoids in sheep and goats. At preliminary surgical operations, these animals are provided with: (1) a London cannula on the portal vein for the withdrawal of blood sample without anaesthesia, and (2) an abomasal cannula for introduction of carotene preparations into the digestive tract, thus avoiding the inevitable dilution by rumen contents consequent upon oral administration.

The preparations were in various forms, including colloidal solutions of crystalline β-carotene, the unsaponifiable fraction from red palm oil dispersed in water with or without bile salts, and homogenized spinach (Nestlé). The doses administered varied between 12,000 and 120,000 μg. of carotenoids. Simultaneous blood samples (20 ml.) were drawn from jugular and portal veins before, and at various times up to 24 hr. after administration of carotenoids, and the plasma examined for carotenoids. So far it has

not been possible to demonstrate the presence of carotenoids in any sample. A large dose of vitamin A into a goat's abomasum was followed by a substantial rise in the vitamin A levels in both systemic and portal blood.

Experiments are in progress to check the rate and completeness of absorption of administered carotenoids by providing further animals with terminal ileal fistulae, in addition to the above preparations, so that the residues from the small intestine can be examined for unabsorbed carotenoids.

The Separation of Ketonic and Non-ketonic Sodium Glucuronidates from the 'Sodium Pregnanediol Glucuronidate' of Human Pregnancy Urine. By ELISABETH S. SUTHERLAND and G. F. MARRIAN

Marrian & Gough (1946 *a*, *b*) have recently shown that 'sodium pregnanediol glucuronidate' ('NaPG') prepared from human pregnancy urine contains *c.* 20% of a water-soluble derivative, presumed to be the sodium glucuronidate, of pregnane-3(α)-ol-20-one. They also showed that this ketonic contaminant could not be removed by the usual methods used for the purification of NaPG, i.e. precipitation from aqueous acetone solution by acetone and crystallization from 90% ethanol. The main glucuronide components have now been separated from the 'NaPG' mixture in states approximating to purity by means of Girard's reagent T (Girard & Sandulesco, 1936) under rather special conditions.

Although pregnanediol glucuronic acid is moderately soluble in ether, it was not found to be possible to extract it with ether from the diluted Girard reaction mixture after neutralization in the usual way of nine-tenths of the acetic acid used in the reaction. This is presumably due to the relative strength of pregnanediol glucuronic acid as an acid. However, by employing ice-cold conditions in order to retard the decomposition of the hydrazone of the ketonic compound, and by working as rapidly as possible, it was found that the usual neutralization of the greater part of the acetic acid in the reaction mixture could be dispensed with. In this way a glucuronide fraction containing considerably less ketonic material than the original 'NaPG' was extracted from the diluted reaction mixture with ether. The sodium salt of this fraction was obtained by extraction of the ether with aqueous sodium hydroxide followed by extraction of the alkaline extract with butanol. This material was subjected to two further successive Girard fractionations in the same way when a product was obtained which gave a virtually negative Zimmermann test. This non-ketonic sodium glucuronide was purified by three successive precipitations from aqueous acetone solution by dry acetone. The final product melted at 282–283° (decomp. and evolution of gas) (corr.) and was obtained in a yield of 0·57 g. from 2·04 g. of 'NaPG'. It gave a strongly positive Tollens reaction and the carbon, hydrogen and sodium contents found were in good agreement with those required for a compound of the formula $C_{27}H_{43}O_8Na$.

The ketonic glucuronide fraction was obtained from each of the aqueous phases from the three Girard separations in the following way: the hydrazone of the ketonic component was decomposed by allowing to stand for 1 hr. after acidification with HCl. The mixture was then made alkaline with sodium hydroxide and the sodium glucuronidate extracted with butanol. The three crude ketonic products so obtained were combined and subjected to two further successive Girard fractionations in order to remove as far as possible all traces of non-ketonic glucuronide. This fraction was finally purified by three successive precipitations from aqueous acetone solution with acetone. The product, which gave an intense brick-red colour in the Zimmermann test, melted at 257–260° (decomp. and evolution of gas) (corr.), and was obtained in a yield of 0·13 g. from 2·04 g. of 'NaPG'. It gave a strongly positive Tollens reaction, but the carbon, hydrogen and sodium analyses indicated that it was contaminated with some inorganic material.

100 mg. quantities of the original 'NaPG', the non-ketonic sodium glucuronidate, and the ketonic sodium glucuronidate were hydrolyzed with HCl and extracted with toluene under the conditions used by Marrian & Gough (1946*b*). Quantitative determinations of the 20-ketosteroid and pregnanediol contents of the unpurified toluene-soluble hydrolysis products were carried out as described by these authors and the following results were obtained:

Toluene-soluble hydrolysis product from	Pregnanediol content %	20-Ketosteroid content %	m.p. (corr.) without purification
Original 'sodium pregnanediol glucuronidate'	77	23	211–220°
Non-ketonic sodium glucuronidate	98	5·2	233–237°
Ketonic sodium glucuronidate	23	85	127–147°

The hydrolysis product from the non-ketonic glucuronidate after crystallization from ethanol melted at 236–238° (corr.) and showed no depression of melting-point on admixture with authentic pregnanediol (m.p. 235–237°, corr.). The hydrolysis product from the ketonic glucuronidate after purification by adsorption on an Al_2O_3 column and crystallization from hexane melted at 145–148·5° (corr.) and showed no depression of melting-point on admixture with authentic pregnane-3(α)-ol-20-one (m.p. 147–149·5°, corr.).

The results obtained show: (1) that the non-ketonic glucuronidate fraction obtained is virtually pure sodium pregnanediol glucuronidate; and (2) that the ketonic fraction, although somewhat impure, consists mainly of the hitherto undescribed sodium pregnane-3(α)-ol-20-one glucuronidate.

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The Formation of Kojic Acid (3-hydroxy-6-hydroxymethyl-γ-pyrone) from Ethyl Alcohol by *Aspergillus oryzae*. By Douglas Barnard and Frederick Challenger

Morphological details of the strain of *A. oryzae* employed are given by Challenger, Klein & Walker (1931). The mould was grown upon Kinoshita's medium (*K*) containing 3% glucose; for details see Challenger *et al.* (1929). The well-developed mycelium, thoroughly washed with sterile water, was stored over sterile water for 24 hr. to remove residual glucose and kojic acid. A 1% solution of ethyl alcohol in the same (glucose-free) medium was then added. Kojic acid was detected by the red colour given with ferric chloride and isolated by precipitation of the copper salt and decomposition with hydrogen sulphide. Mixed melting-point determinations established its identity.

Confirmation of these results was obtained by inoculating medium *K*, containing 1·3% ethyl alcohol as sole carbon source, with spores of the mould. On incubation at 32° C. growth was considerable by the 8th day, spore formation occurring on the 12th day. Kojic acid was first detected on the 9th day and isolated as before on the 18th day. The diacetyl and dibenzoyl derivatives were compared with authentic specimens. Two similar experiments using 1·3 and 2·1% ethyl alcohol confirmed these results.

2% of ethyl alcohol appears to be the optimum concentration for kojic acid production using *K* medium. In two experiments a washed mycelium upon *K* medium with 4% ethyl alcohol as sole carbon source produced no kojic acid in 14 days; on distillation of the medium, acetaldehyde was readily detected by odour and converted to the 2.4-dinitrophenylhydrazone. A blank experiment on the same medium gave no acetaldehyde in 14 days.

Sakaguchi (1932), quoted by Prescott & Dunn (1940), found that ethyl alcohol increased the yield of kojic acid produced from glucose by *A. oryzae*. Tamiya (1932) grew the mould on a 2·3% solution of ethyl alcohol but did not obtain kojic acid. Similar negative results are recorded by Katagiri & Kitahara (1933).

Birkinshaw, Charles, Lilly & Raistrick (1931) found that all strains of the *flavus-oryzae* type, found to produce kojic acid from sucrose, also formed ethyl alcohol. They suggested that acetaldehyde might be an intermediate in the formation of kojic acid. Using washed mycelia and 1·3% alcohol-*K* medium we find that sodium sulphite diminishes but does not inhibit kojic acid formation. In one experiment a small amount of acetaldehyde was 'fixed'. 'Dimedone' similarly diminished the yield and in two experiments crystals of the condensation product with acetaldehyde were observed in the medium and isolated. None was formed in the uninoculated medium.

Kojic acid was not formed, however, and no further growth occurred when acetaldehyde sodium bisulphite (1%), paraldehyde (0·5 and 1%) or acetal (1%) in *K* medium were placed under well-grown mycelia and incubated for 28 days. Negative results were obtained with methyl alcohol and ethylene glycol.

A. *Experiments using mycelia*

Concentration of alcohol	First detection of kojic acid (days)	Duration of exp. (days)	Weight of copper salt (g.)	Weight of kojic acid (g.)	Yield on C/C basis (%)
1%	3·5	10	—	0·05	1·5
1·3%	4·0	11	0·6	0·46	10·9
1·3% alcohol added at 7 day intervals Total = 5·2%	4·0	28	3·3	2·4	14·5

Melting-point (° C.)		Diacetyl derivative		Dibenzoyl derivative	
Alone	Mixed	Melting-point	Mixed	Melting-point	Mixed
150–151	150·5–151·5	—	—	—	—
151·5–152·5	151·5–152·5	—	—	—	—
152–153	152–153	—	—	—	—

The authentic specimens of kojic acid melted at 152–153°.

B. *Experiments using spore cultures*

Concentration of alcohol	First detection of kojic acid (days)	Duration of exp. (days)	Weight of copper salt (g.)	Weight of kojic acid (g.)	Yield on C/C basis (%)
1·3%	9·0	18	0·7	0·51	12·4
1·3%	9·0	18	0·68	0·49	12·0
2·1%	13·0	21	1·6	1·1	16·7

Melting-point (° C.)		Diacetyl derivative (° C.)		Dibenzoyl derivative (° C.)	
Alone	Mixed	Melting-point	Mixed	Melting-point	Mixed
152·5–153	153	101–102	101–102	132–133	132–133
152–153	152–153	—	—	—	—
152–153	152–153	101–102	101–102	—	—

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Gastric Ulcer Production in the Isolated Mucosa. By R. E. DAVIES and NORAH M. LONGMUIR

When gastric mucosa secretes acid, an equivalent amount of alkali is also produced which is at once neutralized by CO_2 (Davies, 1946). The present paper deals with the question whether tissue damage might occur in the absence of an adequate supply of CO_2.

Frog gastric mucosae were incubated in either bicarbonate saline gassed with 5% CO_2 and 95% O_2, or in phosphate saline gassed with O_2. The technique was as previously described (Davies, 1946). In both series there was little or no acid production without histamine, and no ulcers were formed. With histamine (1 mg. %) the mucosae showed a wide range of responses, producing secretions in varying amounts with acidities between 0 and 0·1 N. Since diffusion is slow, the concentration of histamine near the oxyntic cells would be much smaller than 1 mg. %. Smaller quantities of histamine produced smaller responses.

In bicarbonate saline the Q_{HCl} sometimes rose to twice the Q_{O_2} without causing visible damage to the tissue. In phosphate saline, however, when the Q_{HCl} rose above the Q_{O_2}, then tissue disintegration occurred with perforations in very active mucosae after 4–6 hr. Histamine-resistant mucosae were never ulcerated. Whilst responsive mucosae in bicarbonate saline produced clear colourless secretions, in phosphate saline the secretions were opaque with cell debris. As expected, the difference between the 'free' and the 'total' acidity was much greater in the secretions from damaged than from undamaged mucosae due to the buffering effect of this cell debris.

These experiments demonstrate that ulcers can be produced in gastric mucosae by interfering with the normal neutralization of the alkali by CO_2. Possibly a combination of factors causing stimulation of active acid secretion but not providing sufficient CO_2 to neutralize the alkali passed into the blood may be operative in the causation of some types of human gastric ulcers.

The above-mentioned fact that the Q_{HCl} can rise much higher than the Q_{O_2} disproves the theories of Bull & Gray (1945) and Conway, Fitzgerald & Walls (1945) who postulated that the hydrogen ions come from acids such as lactic or pyruvic formed from glucose during respiration. In this case the Q_{HCl} could never be more than one-third the Q_{O_2}.

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Effect of Titration Time in Potentiometric Dye Titration of Apparent Vitamin C. By F. WOKES

The potentiometric method of Harris, Mapson & Wang (1942) for estimating vitamin C has been applied with certain modifications to the estimation of different constituents of apparent vitamin C, some of which may be found in foods. The rate of reaction with the dye has been followed by e.m.f. readings taken every few seconds at room temperature and pH 0·6–0·8, and the dye titration value (D.T.V.) has been found to vary widely with the titration time (T.T.). When D.T.V. is plotted against log T.T. the curves become linear and parallel. Hydroxytetronic acid, dihydroxymaleic acid, iso-ascorbic acid and diketogulonic acid give curves almost coinciding with the curve for ascorbic acid, indicating a similar rate of reaction with the dye under the experimental conditions. Curves for reductic acid and glucoreductone show rates of reaction respectively a half and about a quarter of that of ascorbic acid. Nevertheless, the slope of the curves shows that in a

mixture of glucoreductone and ascorbic acid the titration of the latter will not be completed until an appreciable amount of glucoreductone has been titrated. Hence, in methods where correction is attempted for the presence of interfering substances by extrapolating back to zero time, due allowance should be made for the presence of glucoreductone or reductic acid. Curves obtained with different food materials containing apparent vitamin C (after destruction of the true vitamin C with HCHO at pH 4·5) indicate that the former is generally of the reductic acid type in fruit juices but of the reductone type in walnuts and dehydrated vegetables. In walnuts however glucoreductone does not appear to be present as such (Wokes, 1946). The rate of reaction is affected by the pH which did not vary sufficiently to affect the above findings, which are in general agreement with those obtained by the 'continuous flow' method (Harris & Mapson, 1944).

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Clinical Assay of Vitamin P. 1. Relation between Response and Initial Capillary Resistance. By H. SCARBOROUGH and F. WOKES

In studies on groups of boys on a controlled diet in an institution it has been found that the magnitude of the response to preparations containing vitamin P is largely conditioned by the initial capillary resistance (C.R.) as measured by a negative pressure method in each of two 'standard' areas on the front of the forearm (Scarborough, 1941). When the response, measured as direct increase in mm. Hg suction, is plotted against the initial C.R., rather widely scattered points are obtained but it is clear that the response is greater the lower the initial C.R. When log C.R. is used the relation between response and initial C.R. becomes uniform and more nearly linear. Since this relationship is such that it must be taken into account when assaying the potency of vitamin P preparations, a set of factors has been devised for adjusting the response of each individual to a standard initial C.R. Measurements of C.R. separately in the two 'standard' areas give uniformly similar results, the ratio between such measurements averaging 1:1·20 ($r = +0·92$). Since the factors are found to be similar for values in the two 'standard' areas a common set of factors is employed to adjust results in both areas which may be pooled. A C.R. of 300 mm. Hg suction has been chosen as the initial C.R. to which all results are adjusted. Such a value lies in the middle of the working range and is also about the centre of the theoretical range. For some distance above and below this level the relation between response and initial C.R. is linear, hence the factors give the best (i.e. the most accurate) results when employed on data not far removed from 300 mm. Hg. The limits we use for the initial C.R. are from 200 to 400 mm. Hg suction, which range is found to embrace over 70% of the experimental data.

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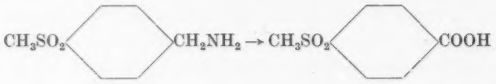
The Inactivation of p-Aminomethylphenyl methyl sulphone (V335) *in vivo*. By R. L. HARTLES and R. T. WILLIAMS

p-Aminomethylbenzenesulphonamide (Marfanil) is inactivated *in vivo* by conversion into *p*-carboxybenzenesulphonamide (Hartles & Williams, 1946) which has no activity against *Staph. aureus*, *Strep. pyogenes* or *C. Welchii*. The V-drug *p*-aminomethylphenyl methyl sulphone is inactivated in a similar manner. When V335 is administered orally to rabbits

it is almost quantitatively excreted as p-*methylsulphonylbenzoic acid* (m.p. and mixed m.p. 265°). The overall reaction is



p-Methylsulphonylbenzoic acid has no action on *Staph. aureus* or *Strep. pyogenes*.

Neither Marfanil nor V335 undergo acetylation *in vivo*, but if acetylmarfanil (*p*-acetaminomethylbenzenesulphonamide) is fed to rabbits it is excreted unchanged and is not deaminized and oxidized as is the free drug.

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The Effect of Testosterone Propionate on the Body Weight of Monkeys. By G. A. Overbeek and M. Tausk

In experiments on three castrated female rhesus monkeys (*Macaca mulatta* Zim.) with a body weight of about 5000 g. we regularly obtained an increase in weight during the daily subcutaneous injection of at least 5 mg. of testosterone propionate (Neo-Hombreol) over a period of 3 weeks or more. The average daily increase after the administration of different dosages is clear from Table 1, 5 mg. being active, 2 mg. probably not. The average daily increase of the three monkeys in altogether seven periods of treatment with 5 mg. was 19·0 g. After each treatment a control period was included during which the animals were either not injected or injected with solvent. The weight then came back to about the initial level. An enormous dosage of cortical hormone (adrenal cortical extract together with synthetic desoxycorticosterone) was inactive.

Table 1. *Average daily increase in weight with the daily injection of testosterone propionate*

Dosage Test. prop. (mg.)	Monkey 1	Monkey 2	Monkey 3
12·5	13·0 (23)	10·9 (170)	—
	16·7 (27)	—	—
5·0	29·5 (44)	27·5 (44)	20·2 (99)
	15·6 (68)	11·3 (68)	19·8 (44)
	—	—	9·3 (68)
2·0	—	—	3·7 (63)
1·0	—	0·5 (63)	—
0·5	1·4 (63)	—	—

The figures between parentheses indicate the number of days of treatment.

Additional observations were the incidental occurrence of a polycythaemia (values as high as 8 millions/mm.^{3} being recorded) a somewhat purplish coloured bloated face, a beautiful shiny fur, and highly increased strength. The blood sugar level was normal. The polycythaemia was not always present, although it occurred at some time in all the monkeys but not necessarily at the same time. The other effects were regularly observed.

In addition we tried whether the weight increase could be inhibited by the simultaneous administration of oestradiol monobenzoate, the dosage of testosterone propionate being 5 mg. (Table 2).

After discontinuing the oestrogenic hormone administration, a period followed during which exclusively 5 mg. of testosterone propionate was given (last line of Table 2).

Table 2. *Average daily increase in weight with daily injection of 5 mg. testosterone propionate and oestradiol monobenzoate*

Dosage oestr.m.benz. (mg.)	Monkey 1	Monkey 2	Monkey 3
0·5	− 6·5 (26)	−14·8 (26)	+2·0 (26)
0·1	+ 6·1 (36)	+ 9·2 (36)	+4·6 (36)
0·05	+ 4·0 (50)	+ 7·3 (50)	+3·0 (50)
0·0	+15·6 (68)	+11·3 (68)	+9·3 (68)

The figures between parentheses indicate the number of days of treatment.

It is clear from Table 2 that in all cases the weight increase was less than after the administration of testosterone propionate alone which makes it probable that the oestrogenic hormone at least partly inhibits this effect of male hormone.

The Inhibition of Esterases by Paludrine. By H. Blaschko, T. C. Chou and I. Wajda

The affinity of paludrine for esterases was studied, as it is known that certain ester-splitting enzymes are inhibited by anti-malarial drugs, e.g. by quinine and mepacrine. Our results are summarized in the following table.

Tissue used	Substrate and concentration	Inhibition in % by paludrine 10^{-3} M	10^{-4} M
Dog's caudate nucleus	6×10^{-3} M-Acetylcholine	22	0
Human red cells haemolysed	6×10^{-3} M-Acetylcholine	49	0
Horse serum	6×10^{-3} M-Benzoylcholine	67	35
Guinea-pig's liver	6×10^{-3} M-Benzoylcholine	94	79
Ox kidney	6×10^{-3} M-Benzoylcholine	0	—
Rabbit's serum	1% Atropine	48	—

Paludrine had little affinity to the cholinesterase of the central nervous system and this is in accordance with its low toxicity. Some of the other cholinesterases, however, were more strongly inhibited by paludrine.

The hydrolysis of both methyl butyrate and tributyrin by extracts of the rabbit's pancreas was unaltered in the presence of 10^{-3} M-paludrine.

The Effect of Ethanol on the Metabolism of Methanol in Man. By L. J. Zatman (introduced by L. P. Kendal)

In 1943 Røe published results of clinical investigations of methanol poisoning in which he gave case reports of some sixteen subjects. Several of these subjects, he noted, had drunk ethanol either simultaneously with or shortly after the methanol and in all such cases the symptoms of methanol poisoning were judged to be much less severe than after methanol alone. Røe therefore suggested that ethanol acts as an antidote in methanol poisoning by virtue of 'the capacity of ethyl alcohol to displace methyl alcohol from the inner surface of cells'. In the present study the effect of ethanol on the metabolism of its methyl homologue has been investigated.

In vitro experiments were carried out with alcohol dehydrogenase prepared according to Lutwak-Mann. In manometric experiments using the ferricyanide technique of Quastel & Wheatley, it was found that the dehydrogenase was able to oxidize methanol at only

about one ninth of the rate for ethanol. When these substrates were presented together to the enzyme, the rate of oxidation of methanol was assessed by estimation of the formaldehyde formed from this substrate by a method based on the one suggested by Wright for the estimation of methanol using a modified Schiff's reagent. No detectable oxidation of methanol took place when ethanol was present in equimolar concentration, and inhibition of methanol oxidation was demonstrable when the molar ratio ethanol to methanol was only about 1 to 16. The inhibition was found to be competitive in type.

If this inhibitory effect is operative *in vivo*, then one would expect a simultaneous dose of ethanol to cause diminished oxidation of ingested methanol and therefore increased excretion of methanol, both in the urine and in the expired air. Experiments on three different human subjects have shown this to be the case as regards urinary excretion. The concentration of methanol in the urine after ingestion of this alcohol (which has been shown by Haggard *et al.*, Yant & Schrenk and Eggleton to follow the blood concentration closely) was much increased when ethanol was taken together with the methanol, the latter being estimated by a method based on the one suggested by Wright.

Various authors have attributed the characteristic toxic effects of methanol to the action of oxidation products of methanol rather than to methanol itself. If their view is correct, the experimental results described above would lead one to expect ethanol to diminish the toxicity of methanol, permitting the excretion in an unchanged condition of a larger fraction of the ingested dose.

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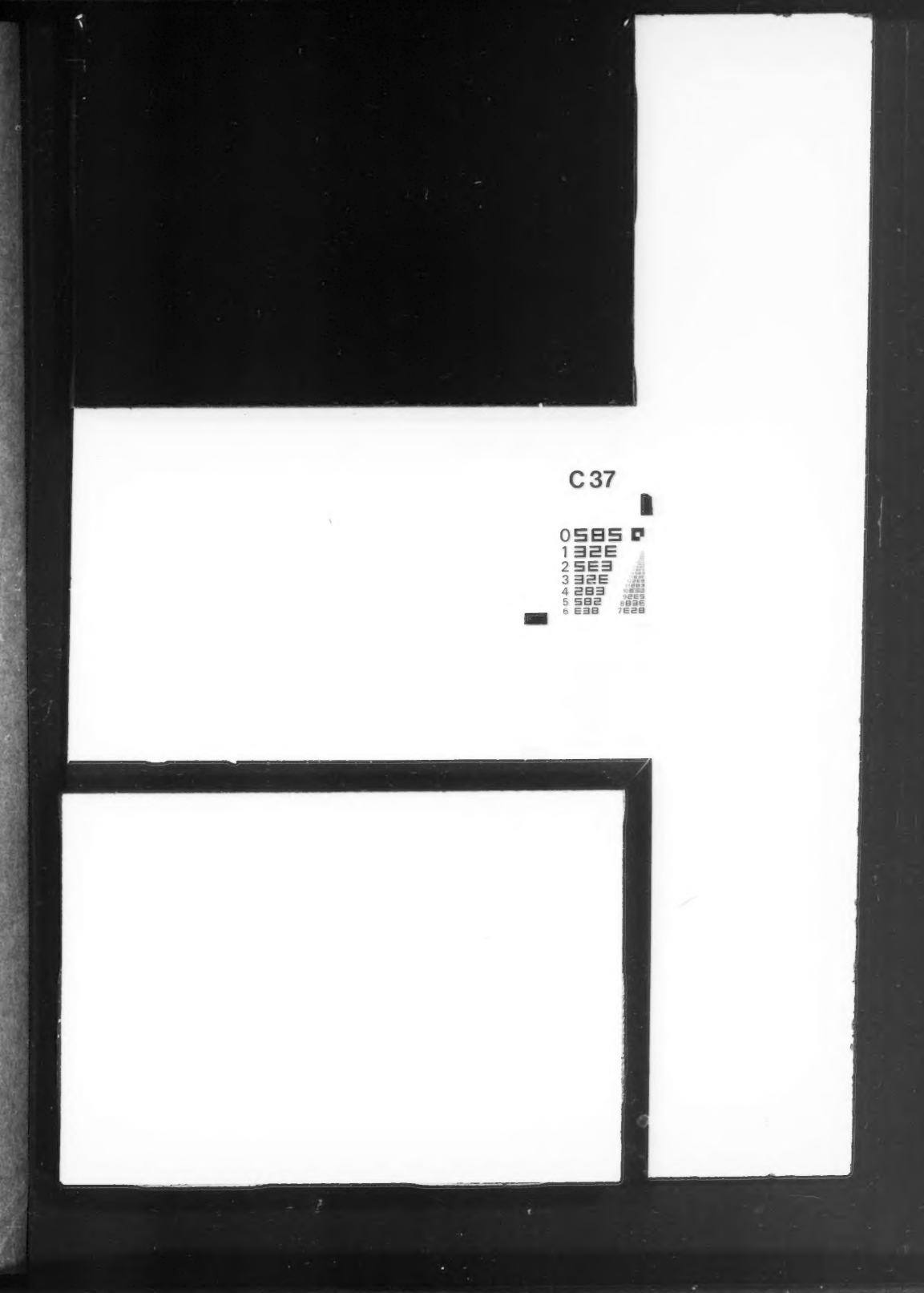
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